VOLUME 15 NUMBER 5

ENDOCRINOLOGY

THE BULLETIN OF THE ASSOCIATION
FOR THE STUDY OF

INTERNAL SECRETIONS

EDITORS

R. G. HOSKINS, Boston

M. O. LEE, Boston H. LISSER, San Francisco

SEPTEMBER-OCTOBER

1931

PUBLISHED BI-MONTHLY BY THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, SUITE 1214 WILSHIRE MEDICAL BLDG., LOS ANGELES, CALIFORNIA
ANNUAL SUBSCRIPTION, $6.00 SINGLE COPIES, $1.25

New Books

ALLERGY AND APPLIED IMMUNOLOGY

By Warren T. Vaughan, M.D. 350 pages with many illustrations and tables. Price, about $3.50. A handbook for physician and patient, on asthma, hay fever, urticaria, eczema, migraine, and kindred manifestations of allergy.

DISEASES OF THE SKIN

By Richard L. Sutton, M.D. 1385 pages, with 1290 illustrations in text and 11 color plates. Cloth, $12.00. New enlarged and revised eighth edition of the standard text on skin diseases.

BEDSIDE INTERPRETATION OF LABORATORY FINDINGS

By Michael G. Wohl, M.D. 324 pages, with 133 text illustrations and 4 plates in colors. Cloth, $6.00. Introduction by Joseph McFarland, M.D. This new work fills the gap between the laboratory and the bedside. It points out the limits of error in the most commonly used laboratory tests.

CLINICAL DIETETICS

By Harry Gauss, M.D. 490 pages, with 150 illustrations. Cloth, $8.00. A text for physicians, students and dietitians. Only those diets for which a rational basis exists have been considered; empiric diets with a few exceptions have been disregarded. Case reports are used freely. A new kind of book.

HISTOPATHOLOGY OF SKIN DISEASES

By Lee McCarthy, M.D., Associate Clinical Professor of Dermatology, Georgetown University, etc. Membre Correspondent Etranger de la Societe Francaise de Dermatologie et de Syphiligraphic, etc. 513 extra large pages, 197 original half-tones, and 54 beautiful color plates prepared by special artists. Price, special binding, $25.00. No expense has been spared in the production of this monumental work. Beautifully illustrated throughout.

CUTANEOUS X-RAY AND RADIUM THERAPY

By Henry H. Hazen, A.M., M.D., Professor of Dermatology, Medical Dept. of Georgetown University; Professor of Dermatology, Med. Dept. of Howard University, Washington, D. C., etc. 160 pages, with 28 engravings. Cloth, $3.00. This book fills a long-felt need for a small, compact, practical, and authoritative work that will aid the physician in the selection of cases for irridation, give safe advice as to technic, and warn against the many pitfalls.

Write us today for our complete catalog of publications. We shall be glad to send any of these books on application. Just say that you saw it advertised in this magazine.

The C. V. Mosby Company—*Medical Publishers*

3523 Pine Boulevard **St. Louis, U. S. A.**

ENDOCRINOLOGY

THE BULLETIN OF THE ASSOCIATION FOR THE STUDY OF

INTERNAL SECRETIONS

Volume 15, Number 5 SEPTEMBER-OCTOBER, 1931 Serial No. 79

THE CONSIDERATION OF SOME OF THE GLANDS OF INTERNAL SECRETION FROM A CHEMICAL VIEWPOINT*

EDWARD C. KENDALL

From the Section of Biochemistry, Mayo Foundation

ROCHESTER, MINNESOTA

The Association for the Study of the Internal Secretions has been in existence for fifteen years. It has passed through its infancy without signs of thyroid deficiency and its growth curve indicates a normal, if not over active, pituitary. It has maintained a balanced proportion in its development so that neither the clinician nor the physiologist have induced what could be described as a neoplasm of malignant type. Its life blood is regularly delivered at constant pH through the pages of our journal. As we examine it today there is no sign of high blood pressure nor any evidence of arteriosclerosis. From the very beginning it has been composed of two groups: those who work either as clinicians or surgeons directly with patients and those who work in laboratories to determine the physiological significance and the chemical nature of the group of glands which deliver an internal secretion. It has become a custom for your president to give an address at the annual meeting of the Association. I shall continue this custom and shall endeavor to present certain aspects of endocrinology from the viewpoint of the chemist. The sequence of events which has almost invariably occurred in the study of each of the ductless glands has been first, the contribution of the surgeon who has demonstrated the end results produced by the absence of the essential constituents of the glands; this was followed by the contributions of the clinician and physiologist who have shown the beneficial effects of substitution therapy. This in turn has been followed by the work of the chemist who has concentrated the active fraction until the substance could be isolated in pure form or its identity made certain. Through the agency of organic chemistry the last step has been consummated and the active constituents of two of the ductless glands have been prepared synthetically.

Up to the point where the chemist has carried out the necessary concentration of the active constituent the results have been of empirical nature. Often times statistical treatment has been required to determine whether the material under investigation could throw the average result one side or the other of the median. The chemist, however, obtains his

*Presidential address delivered at the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 8, 1931.

answer in terms of percentage composition, the ratio of nitrogen to sulfur or the percentage of iodine in a crystalline compound.

Someone has said that it gave him a great deal of satisfaction to know that the active constituent of the islands of Langerhans was not fractionated for the first time by a chemist. It is true that insulin was discovered by investigators trained primarily in surgery and physiology, but since that first dramatic result the essential contributions to our knowledge of insulin have been made in chemical laboratories and no surgeon would now step into the field in the hope that he could contribute to our knowledge of the essential nature of the crystalline polypeptid.

It is necessary that the dependence of each of these groups on the other be recognized. It is not a matter of rivalry but of cooperation. Although some surgeons may be prepared to work in a chemical laboratory and although some chemists may be able to use the scalpel or make a venipuncture, the time will soon come, if it has not already arrived, when investigations carried on by one man will cease to be profitable. It is interesting to note that there is not a single instance in which the active constituent of any of the ductless glands has been fractionated, isolated in pure form, identified and synthesized by any one investigator or even in any one laboratory, and as the interest and number of workers in this field increase the probability that this will ever be done becomes more and more remote. A few weeks after the discovery of insulin was announced the number of papers which were published concerning the method of separation and properties of insulin began to rise on a steep curve and the volume of publications which one can see in any abstract journal emphasizes the large body of investigators that are engaged in a study of this substance. This is equally true of the other ductless glands.

After the essential and primary contribution of the surgeon and physiologist have been made, their work closes a chapter and the following pages are written by the chemist. However, the chemist's work is not finished when the active constituents of the ductless glands under investigation have been separated in crystalline form, identified and synthesized. Although this portion of the work may be the most interesting and may carry the most sensational consequences, the most significant contribution of the chemist consists in the quantitative measurement of the chemical reactions which are controlled by the compounds under investigation. In this respect the chemist occupies a unique position in the study of endocrinology.

In a very real sense the chemist may feel that the future study of the ductless glands rests on his shoulders. The demonstration that certain substances must exist within the various ductless glands is a stimulating challenge. The difficulties involved, however, and one essential which is sometimes overlooked are clearly indicated by the investigations of the two ductless glands which have been most completely studied, the thyroid and the suprarenal.

The chemical investigation of the glands of internal secretion began when Emil Baumann was requested to carry out a study of the thyroid

gland. You are all familiar with the details of this work, but I wish to remind you that he discovered the presence of iodine in the gland in 1895. Within a year Drechsel separated diiodotyrosine from sponges. In 1907 Nurnberg concluded that the iodine in the thyroid was combined either with tryptophan or tyrosine. Twenty years elapsed from the work of Baumann before the iodine containing compound was separated in crystalline form and nineteen years were required to answer Nurnberg's question as to which of the two amino acids carried the iodine. Diiodotyrosine was separated from the thyroid thirty-three years after Drechsel first isolated this compound from sponges. These three questions have now been solved but today, thirty-six years after Baumann began his work, no one can say how thyroxine brings about its effect. These long lapses between the significant contributions emphasize the difficulties involved and remind us that the work has been carried on by human endeavor.

The results of research are a summation of the imagination, the ability and the persistence of those investigators who carry on the work.

Although it is impossible to define the chemical reactions which are controlled by thyroxine a great deal is known concerning the activity of the thyroid gland and the probable course which is followed for the elaboration of its active agent. We now know that the total iodine in the gland can be fractionated into two portions. The work of Tatum, van Dyke, Pearse, Williamson and Cunnington and some work which has been carried out in my own laboratory have clearly shown that the thyroid gland must build up the thyroxine molecule through several distinct steps. The iodine contained in the cells, although it constitutes but a small proportion of the total iodine, has been found to be biologically inactive when tested for its power to accelerate the metamorphosis of tadpoles. In every case, however, the iodine which was in the acini was highly active in its effect on the metamorphosis of the tadpole. The identity of the compounds contained within the cells and in the acini has not been ascertained. However, it has been shown that diiodotyrosine is not the only iodine containing compound which is found within the cells. Fractionation of the iodine compounds in the cell from those in the acini does not fractionate the iodine containing compounds into the two substances, thyroxine and diiodotyrosine.

Chemical separation of the iodine compounds without respect as to whether they are contained in the acini or in the cells results in the separation of about two-thirds of the total iodine in the form of diiodotyrosine. Whether or not the remaining one-third is all in the form of thyroxine has not been shown by the actual separation of this amount of thyroxine from the gland. There are many possible explanations which can be made to account for the discrepancy in the amount of thyroxine which can be separated and the amount which is indicated by chemical fractionation. We may leave this question feeling confident that within a relatively short time satisfactory evidence will be secured.

A large amount of work has been carried out with the object to determine the essential groups in thyroxine which are responsible for its physio-

logical activity. At the recent meeting of the Federation of Biological Societies, Rapport gave the results of the determination of the effect of four compounds on the basal metabolism. These compounds were tyrosine, diiodotyrosine, thyronine (which is the same as thyroxine except that the two atoms of iodine on the oxyphenyl ring have been replaced with hydrogen) and thyroxine. In a general way the effect on the basal metabolism increased in the order of the substances mentioned, but actually the difference between thyroxine and all the other compounds both in respect to the amount used and the length of the effect on the basal metabolism was so marked that it did not constitute a quantitative difference; the effect was qualitatively different. That is to say, the specific effect of thyroxine can not be produced by any other substance known. This fact is also recognized as a result of the experiments in substitution therapy in patients with hypothyroidism. The thyroxine molecule is not simply a more efficient agent that tyrosine or diiodotyrosine, its effect is specific and unique.

When a patient with hypothyroidism is treated with crystalline thyroxine one is impressed by the extraordinary number of different physiological effects which are produced. The results are not confined to any one organ or system of organs and it is difficult to explain how the single substance can bring about such a marked effect in so many processes which are essentially independent of each other.

The administration of thyroxine to animals and to the larvae of amphibia have shown two distinctly different effects: one is on the basal metabolism, the other on the rate of metamorphosis. In its effect on the basal metabolism it is necessary to assume that the thyroxine molecule brings about its effect by reason of its chemical structure. There is no indication that the iodine is split off from the molecule and from the time relations of the response of the animal organism it seems probable that thyroxine functions as a catalyst. In the effect on the metamorphosis of amphibian larvae it seems highly probable that the result is not brought about by an increase in the rate of metabolism. The process apparently involves a specific reaction induced by iodine, since the same effect can be brought about by other compounds which contain iodine and these substances do not effect the basal metabolism. The effect of the acetyl of thyroxine on the animal organism is of particular interest. In amounts comparable with the physiological dose of thyroxine the acetyl derivative does not produce any effect and yet the acetyl of thyroxine will induce the metamorphosis of the tadpole. This result suggests that the thyroxine molecule cannot be utilized in the animal organism unless its amino group is free. It can be conclusively shown that in the thyroid gland thyroxine is firmly bound within the protein molecule and no evidence has been secured which indicates that it is split out from the protein molecule when it is utilized by the animal organism. Therefore, it seems highly probable that the active functioning form of thyroxine is a complex group which contains other amino acids. One is, of course, reminded of the complex nature of insulin. This polypeptid loses all physiological activity if the group of amino acids is altered in

any way. The pituitary hormone does not appear to be a simple single substance but a complex group of several molecules. Glutathione is still another example of an active tripeptid. By analogy, therefore, we may feel confident that the thyroxine molecule brings about its physiological effect only after it has been incorporated and that it serves as one unit of a more complex group of molecules.

The incorporation of the thyroxine molecule into a larger group appears to confer on the molecule its unique power to affect the basal metabolism. Pure crystalline thyroxine can be injected intravenously even in large amounts without the immediate production of any physiological effect which can be measured. There is a distinct delay in the development of the physiological response and it appears to be significant that the administration of desiccated thyroid is also followed by a delay in the physiological manifestations.

A review of our knowledge which bears upon the problem of the chemical reactions which are influenced by thyroxine permits us to suggest two hypotheses: the first is that thyroxine acts merely as a mobilizing agent and that the breakdown of substances which are destined to furnish fuel for metabolic processes is controlled by the activity of thyroxine; the second hypothesis is that it acts as a catalytic agent and is involved in oxidative processes. Today it is impossible to exclude either one of these hypotheses and it seems highly probable that parts of each will be required to explain the physiological activity of thyroxine.

It would appear of significance to determine the least complex portion of an animal organism which can still manifest a response to the administration of thyroxine. If thyroxine is given to an animal organism the response is striking and can be measured quantitatively. If a slice of muscle tissue is used the addition of thyroxine to a solution in which it is suspended does not alter the rate at which this portion of tissue can take up oxygen and give off carbon dioxide. Somewhere in the scale of complexity between these two limits it should be possible to demonstrate the action of thyroxine. It has recently been shown that heart muscle can be affected by the presence of thyroxine; its rhythm is markedly influenced. This finding does not supply information which can be expressed in terms of anatomy but it does indicate that some degree of activation of the muscles by nerve impulses is essential in order to demonstrate a physiological response to the thyroxine molecule.

The most important single problem associated with the activity of the thyroid gland which has not yet been solved is concerned with the so-called goiter crisis which occurs in patients with a severe type of exophthalmic goiter. One attractive hypothesis is that the thyroid gland elaborates an abnormal secretion and the toxic symptoms are due to the abnormal product which is produced in the goiter. Because of the striking relief produced by the administration of iodine to patients with exophthalmic goiter it seemed possible that the toxic agent was thyroxine which was not completely iodized. It now seems improbable that any derivative of thyroxine

can be an abnormal agent which is responsible for the toxic symptoms.

The formation of the thyroxine molecule from two molecules of diiodotyrosine involves the removal of the three-carbon side chain from one of the molecules of diiodotyrosine. This reaction is essentially one of oxidation and it seemed possible that under abnormal conditions the gland might carry out the oxidation of diiodotyrosine with the formation of diiodoquinol and diiodoquinone, provided the oxyphenyl group was not attached to tyrosine. Some experiments have been carried out in my laboratory concerning the toxicity of diiodoquinol and diiodoquinone. These substances are not capable of producing any physiological effects which suggest that they are ever formed in significant amounts by the thyroid gland.

In the search for the essential chemical nature of the toxic agent which produces the symptoms in exophthalmic goiter there is, of course, the possibility that one or more of the other endocrine glands may be directly involved. With the rapid advance in our knowledge of the pituitary and suprarenal glands it seems highly probable that a much clearer viewpoint concerning the etiology and treatment of the toxic manifestations of hyperthyroidism will soon be secured.

The second gland which has been thoroughly investigated in so far as its chemistry is concerned is the suprarenal. Chemical investigation began soon after Baumann's work on the thyroid. The details of the work on epinephrin are familiar to you all, but it is interesting to note the time which has elapsed from the first investigation of this gland.

It is a long way from the first crude physiological tests for the presence of epinephrin to our present abundant knowledge concerning this remarkable compound. Before its isolation in crystalline form its presence was recognized by sniffing a few drops of a solution up the nose; this was the most useful test for the compound. The separation of the substance in crystalline form opened wide the doors for both the chemist and physiologist. Ever since its isolation the chemist has been busy with the preparation of a large number of closely related compounds and the pharmacologist has secured important information concerning the relation between the chemical structure and physiological activity of the various compounds. At first it was believed that epinephrin was the only active agent of the suprarenal gland and all efforts to separate any other physiologically active substance from the gland resulted in failure. However, the patient afflicted with Addison's disease was not brought back to normal by the administration of epinephrin and animals, after complete suprarenalectomy, died whether or not epinephrin was administered. This situation produced a problem of major interest and importance. The surgical removal of three-fourths of the cortical tissue in an animal was not followed by death. If the remaining portion of the gland was removed death ensued usually within one week. Prostration, muscular weakness, decrease in the basal metabolic rate, nausea, drop of body temperature, low blood pressure, decrease in the blood volume and marked changes in the distribution of water in the body were the symptoms noted at the time of death. If the

symptoms of suprarenal deficiency were due to the absence of a substance elaborated in the suprarenal cortex then it should be possible to prepare an extract of the cortex which would save the life of animals after suprarenalectomy and of patients with Addison's disease. Needless to say, this problem was taken up by many laboratories. From time to time encouraging results were reported but further investigation in each case revealed the fact that the physiological response had been brought about by the well known substance, epinephrin. During this interval the fact that epinephrin was not essential for life became fixed in the minds of all investigators. A leading physiologist at a meeting of the American Federation of Biological Societies in 1920 asked the question, whether anyone could point out any physiological reaction for which the presence of epinephrin was essential. Epinephrin was regarded as a substance used in emergency and its strongest champions were unable to establish conclusive evidence that it was essential for any known physiological process.

The obvious association between the suprarenal cortex and processes of oxidation in the animal organism suggested to Szent Györgyi that the active agent of the cortex was probably concerned with biological oxidation. After several years of research Szent Györgyi isolated a substance from the cortex which he named hexuronic acid. This substance is present to the extent of about 0.10 per cent in the suprarenal gland. It is readily oxidized by air and its isolation in pure crystalline form is one of the outstanding triumphs of biological chemistry. Szent Györgyi has shown that hexuronic acid is widely distributed in nature, that it is intimately concerned in biological oxidation in plants and that in its presence epinephrin is maintained in the reduced form until all hexuronic acid has been oxidized. Hexuronic acid is one of the most active reducing agents known.

The striking chemical properties of this substance and the fact that it is present in the suprarenal cortex appeared to justify an investigation which had as its object the isolation of hexuronic acid in large amounts for clinical and experimental use. Such a method has now been perfected and results will be reported at another time concerning the significance of this compound in the body.

Szent Györgyi found that although hexuronic acid possessed striking chemical properties, and although it will act as an essential component in a system of phenol and oxydase, still it had very little, if any, effect upon the course of Addison's disease and it did not prolong the life of animals after the suprarenal gland had been removed. As a result of these researches Szent Györgyi suggested that the suprarenal cortex elaborated still another compound.

During the time which was required to develop the method for the isolation of hexuronic acid on a large scale I have made attempts to test out the hypothesis of Szent Györgyi and to see whether or not a substance could be isolated from the cortex which had an effect on biological oxidation. I had hoped to present the results of this investigation at this meeting but shall reserve the report for future publication. While this work

was in progress several publications appeared in rapid succession from two laboratories, that of Hartman and coworkers at Buffalo and that of Swingle and Pfiffner at Princeton. Through a series of brilliant researches these two groups of investigators have removed much of the mystery of the suprarenal cortex. In a convincing manner they have placed substitution therapy for suprarenal deficiency on a firm basis. The chemical investigation has been carried to the point where the solubilities and stability of the compound are clearly established. The separation of the material in crystalline form and its synthesis is only a matter of time.

The active constituents of all the other ductless glands must still be described by their anatomical names except for the two substances, insulin and theelin. The most significant property of insulin is the fact that any alteration of the amino acids which comprise the polypeptid results in the destruction of its physiological activity. This is in striking contrast to the compounds epinephrin and thyroxine. These two molecules can be isolated, free from all other compounds, and still the body is able to build them up again into their active functioning forms. Sufficient is known of the structure of insulin to make it improbable that any hitherto unknown substance is present which has not been isolated. It is remarkable that the animal organism can form a group of the animo acids into a unit which has the unique properties of insulin.

The story of the isolation of theelin will some day be a "best seller" in the Annals of Biochemistry. Starting in with a drastic treatment of the follicular fluid Doisy and coworkers have passed through many stages which have finally resulted in the isolation of a crystalline compound. Its molecular weight has been determined, the form in which the oxygen is present is known, and already some of the degradation products have been studied. The final identification and synthesis of theelin may be regarded as a certainty.

At the fifteenth anniversary of the founding of this Association the chemist's contribution to endocrinology can be summarized in the statement that the essential constituents have been separated in crystalline form from four of the glands of internal secretion. Two of these active constituents, thyroxine and epinephrin, have been identified and synthesized and two, hexuronic acid and theelin, have been crystallized and their essential natures established, but their exact structure has not been determined. One wonders how many more of the active constituents can be added to this list fifteen years after this meeting, and one wonders further whether at that time the biological chemist will have explained in terms of chemistry how any one of the glands of internal secretion brings about its effect.

I will close with a quotation from Seneca, written in the first century. This is taken from Vaughan's autobiography, Memories of a Doctor: "Nature does not disclose her mysteries in a moment. We think ourselves initiated, we stand but at her portals. These secrets open not promiscuously nor to every comer. They are remote of access enshrined in the inner sanctuary."

THREE CASES OF ADULT MYXEDEMA IN WOMEN, REPORTED FOR THE PURPOSE OF CALLING ATTENTION TO THEIR WIDELY DIFFERENT SYMPTOMATOLOGY AND CLINICAL FINDINGS:

CASE 1. ASSOCIATED WITH CARDIAC DISTURBANCE. CASE 2. ASSOCIATED WITH ANEMIA. CASE 3. ASSOCIATED WITH MENORRHAGIA.*

H. LISSER, M.D., AND E. M. ANDERSON, M.D.
From the Endocrine Clinic of the Division of Medicine, University of California Medical School
SAN FRANCISCO

INTRODUCTION

Geographical peculiaritics, such as altitude, latitude, climate or geological formation apparently have no influence on the incidence of adult myxedema. Indeed, Falta states that typical myxedema seems to be relatively rare in districts where goiter is endemic, and Dock mentions its infrequency in Derbyshire, the traditional home of goiter in England. Several American writers have stressed the fact that the disease is almost invariably incorrectly diagnosed and the patient passes through the hands of several consultants before myxedema is recognized. The experience of Anders, Plummer and the writers substantiate this. Moffitt many years ago collected 83 cases in California; many are never reported and many more are never recognized. The disease is probably scattered fairly evenly throughout the world, and impressions of preponderance in certain localities are apt to originate from the fact that a few physicians in these regions have been sufficiently alert to have collected many cases and placed them on record.

All authorities agree that adult myxedema affects women more often than men, the proportions given varying between 4 to 1 and 7 to 1. Adult myxedema, as the name implies, is the clinical syndrome resulting from a deficient thyroid function in adult life, originating after adolescence and at a time when osseous development has been completed. In adult myxedema the skeleton is that of a normal adult. Indeed this constitutes the fundamental distinction from childhood myxedema which in all other respects is very much the same disease. If osseous retardation is detectable in an adult who likewise shows evidence of myxedema, the onset of the disease can be traced back to childhood, and the case is not to be interpreted as a true example of adult myxedema. Of the 89 cases reported by Boothby from the Mayo Clinic, 73 were between thirty and sixty years of age. It is a disease of middle life and the greatest incidence is found between thirty-five and fifty years.

*Read before the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 8, 1931.

It might be well to sketch briefly the customary and more conspicuous symptoms of myxedema. Sensitiveness to cold is important and highly characteristic. This consists of an unbearable chilliness of the entire body, rather than merely "cold hands and feet." The patient ordinarily wears extra clothing; uses more bed coverings at night than the average individual; and "seriously objects to room temperatures comfortable to healthy people" (Boothby, 24). This subjective feeling is confirmed by a markedly subnormal temperature, sometimes as low as 35° C. (95° F.). Incidentally, this symptom is diametrically opposed to the intolerance of heat displayed by the hyperthyroid patient. It is also significant that the first sign of improvement gratefully acknowledged by the patient in response to treatment with thyroid substance is a sensation of "thawing out," an increased feeling of warmth. Mental and emotional lethargy is also a characteristic symptom. Difficulty of concentration, progressive loss of memory, indecision, and loss of initiative, laziness, listlessness, apathy, somnolence, and a slow, monotonous speech are all expressions of a lowered metabolic level. Some gain in weight despite an indifferent appetite is often noted; stubborn constipation is not unusual. These symptoms are likewise the direct antithesis of the loss of weight, despite a ravenous appetite and high caloric intake, which characterizes hyperthyroidism. Puffiness of the skin and mucous membranes is an outstanding clinical feature; this includes, ordinarily, swollen, baggy lower eyelids with narrow palpebral apertures, which so often confuse the patient and physician into suspecting Bright's disease. Lips and tongue and mucous membranes of nose, pharynx and larynx are likely to be thickened by myxedematous infiltrations, a condition frequently accounting for impaired hearing and a low-pitched, thick and leathery voice. The puffy, non-pitting mucilaginous pseudo-edema of the skin and subcutaneous tissues produce a feeling of tightness of the hands and heaviness of the limbs. The gait is slow and clumsy. The skin is apt to be dry, rough, cold, and of a doughy consistency. These patients ordinarily do not perspire. The supraclavicular fat pads, of rubbery firmness, are quite characteristic and occur in no other condition except in the milder forms of hypothyroidism. A nuchal fat pad almost like a hump is often seen. The hands are short and plump, the fingers thick and clumsy and suggest the appearance of mittens (Ewald). There may be bracelets and anklets of myxedematous infiltration, blunting the normal contour of the wrists and ankles. The lower legs may appear tubular. Loss of scalp and body hair is not unusual; moreover, that which remains is apt to be dry, coarse and brittle. Thinning of the outer halves of the eyebrows often has been stressed as characteristic of hypothyroidism.

Although most of the symptoms just described were elicited in the anamnesis or recognized in the course of the physical examination, in none of the three cases to be reported did they constitute the dominating or motivating reason for which the patient sought the physician. It is this emphasis on other factors, not necessarily unique or characteristic of

myxedema, that actuates the writers to report the story of these three patients. There would, after all, be no particular value in merely adding three more cases of adult myxedema to the extensive literature on the subject. It is important, however, to call attention to the fact that the outstanding symptom of which each patient complained and which, in each case, was utterly different, might very well have led physicians astray and left the patients unimproved (and in one case unnecessarily operated upon), had not the underlying common denominator been discovered.

Case I—Adult Myxedema Associated with Cardiac Disturbance
(Referring to Mrs. B. R.; U. C. O. P. D. No. 166130)

A woman of fifty-one years first consulted us November, 1928, in the midst of an attack of paroxysmal tachycardia. She stated that her heart had been beating rapidly for the previous 8 hours. The heart rate was found to be 180 per minute. Vagal pressure produced a perceptible slowing of the heart rate, but did not terminate the attack. An electrocardiogram was taken at once. (See electrocardiogram I in Figure 1.) The rate was 168 per minute. One of our cardiac specialists, Doctor John J. Sampson, provided the following interpretation: "Paroxysmal nodal tachycardia. The P waves are inverted and succeed the R waves, which is probably partially responsible for the S-T interval depression. The T waves are inverted 1.5 mm. in I, 2.5 mm. in II and 1.0 mm. in III. There is no abnormal axis deviation. This record is not inconsistent with nodal escape from S-A bradycardia as associated with myxedema." In view of the extraordinary changes which subsequent electrocardiograms displayed, further comment on this tracing will be reserved until later in the narrative.

The patient had experienced many such attacks of palpitation and rapid heart action during the previous 17 years, the first one having occurred at the age of thirty-four following the birth of the youngest of 4 sons. It is proper to record that the patient was confined to bed for six weeks at the age of fourteen because of "inflammatory rheumatism." It is questionable whether this ailment affected her myocardium; in any event, she was married two years later and passed through 4 apparently normal pregnancies. Seemingly her endocardium was not permanently damaged if at all, since no evidence of a valvular lesion was detected at the age of fifty-one, nor at any time during the two subsequent years that she has been under frequent observation. So far as the etiology is concerned, we are forced to rely on the patient's story; and so far as she is aware the first cardiac episode dates back to the age of thirty-four.

Otherwise there seems to have been nothing of significance in her childhood, adolescence, or earlier adult life which might be construed as bearing on her present illness. Menstruation began at eleven and ceased at forty-two, but beyond severe hot flashes since the menopause, nothing abnormal of an endocrine nature was elicited until the patient was about fifty years of age.

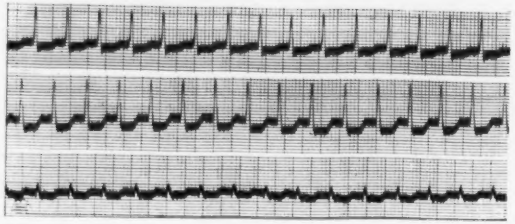
Swelling and puffiness of the face and eyelids, fingers and feet, especially annoying in the morning and receding somewhat during the day, began to attract the patient's attention about one year before we saw her. There occurred, coincident with these "myxedematous infiltrations," a striking alteration in the pitch and quality of her voice, the tones having become hollow, rasping, and of a leathery character. Her appetite was poor, and she had chronic constipation. She described her feet as "frozed to death;" she rarely perspired. On the other hand, the patient had not noticed any changes in the quantity or texture of her hair; indeed, her eyebrows were heavy, rather than scanty.

Soon after the first electrocardiogram was taken, the patient was aware of a sudden slowing of the heart ("it almost stopped"). A second tracing was made 6 days later. The cardiologist was prepared for a diminished heart rate, but was rather astounded to find it as low as 50 per minute (see electrocardiogram II in Figure 1). "S.—A bradycardia is present. The same degree of T inversion exists as previously recorded. There is a left axis deviation. U waves are present, and the S waves are slurred. This is consistent with change in the chamber size at the slower rate than previously recorded."

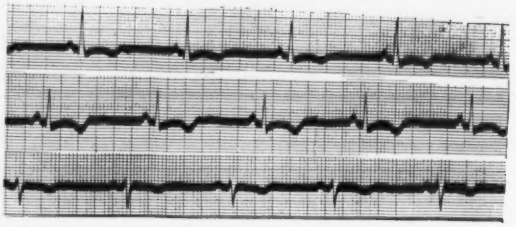
The period of cardiac excitement having ceased, the physician's attention was directed to the patient's puffy features, puffy fingers, brittle nails, dry,

FIGURE 1.

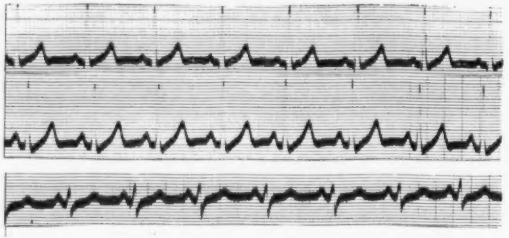
ELECTROCARDIOGRAMS FROM CASE 1. ADULT MYXEDEMA ASSOCIATED WITH CARDIAC DISTURBANCE.



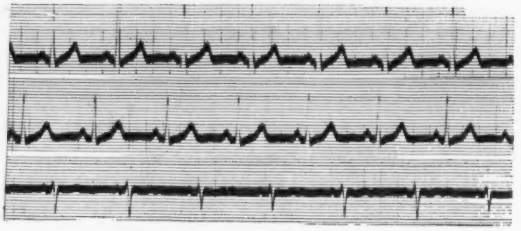
Electrocardiogram I. November 27, 1928. Rate 168. Paroxysmal nodal tachycardia. Depressed S-T intervals. Flattened T waves.



Electrocardiogram II. December 3, 1928. Rate 40. Inversion of T waves in all three leads.



Electrocardiogram III. June 12, 1929. Rate 79. T waves upright in all three leads after 6 months of thyroid administration.



Electrocardiogram IV. September 30, 1930. Rate 72. T waves upright.

scaly skin, deep hollow voice, lack of animation, slow cerebration, and monotonous speech. Determination of the basal metabolic rate seemed to be in order and on December 7, 1928, this was found to be 17 per cent minus. On this same day one grain of desiccated thyroid substance three times daily was prescribed. Quinidine sulphate was also given in 3 grain capsules 3 times a day.

A marked change was noticeable 2 weeks later. The patient had lost 12 pounds, felt warmer, less drowsy, was more attentive, and spoke in a clearer voice that had lost much of its huskiness.

A teleoroentgenogram was taken at this time and showed the heart to be about normal in size, possibly slightly enlarged to the right. The aortic shadow was slightly denser than normal, interpreted as probably due to sclerosis. The blood Wassermann reaction was negative. The attending physician, for the sake of completeness, ordered many other laboratory tests which are not recorded in this narrative since they were all either negative or irrelevant.

Figure 2. Case I. Adult myxedema associated with cardiac disturbance (see Figure 1 for electrocardiograms). Note myxedematous puffiness of face, especially under lower eyelids; note narrow palpebral aperture with sleepy expression. Basal metabolic rate 31 per cent minus.

This patient was not seen in the Endocrine Clinic until toward the end of April, 1929. During the preceding five months she had received decreasing amounts of thyroid substance, because of frequent mild spells of palpitation and at least two severe attacks of paroxysmal tachycardia. During this time her pulse rate had varied from 62 to 150 per minute. The dose of desiccated thyroid substance had been reduced, by February, to one-fourth of a grain every other day.

It was obvious to us when we saw her first, April 25, 1929, that she was still outspokenly myxedematous. This was confirmed by another basal metabolic rate (May 1, 1929) which proved to be 31.1 per cent minus. The patient's appearance may be visualized from Figure 2. Thereupon she was placed on a dose of 1 grain of Armour's thyroid substance twice daily. Because of frequent flashes of heat, 5 grains ovarian substance was prescribed 3 times daily.

The patient was hardly recognizable one month later (See Figure 3.) The change in appearance was quite remarkable; the puffiness had entirely disappeared, and speech, movements and cerebration were much livelier. Incidentally, it may be noted that a slight vaginal flow had appeared twice in the previous two weeks, whereas no such phenomenon had occurred in the previous nine to ten years. There was also a return of sexual desire. By June 12, 1929, the basal metabolic rate had been elevated to 9.7 per cent minus. This was to be expected, but we were not prepared for the astonishing change in her electrocardiogram. The T waves which previously had been inverted in all three leads, were now upright. (See electrocardiogram III in Figure 1.) "The rhythm is normal. The T waves are 5 mm. high in I and II and 1.5 mm. in III. The R waves are 18 to 20 mm. in I and II respectively, as compared to 10-12 mm. on the previous record. The S waves are still slurred. There is no axis deviation. This is consistent with increased metabolism."

Figure 3. Case I. Compare with Figure 2; note disappearance of myxedematous puffiness and alert expression, consequent to thyroid therapy. Basal metabolic rate elevated from 31 per cent minus to 9.7 per cent minus.

On September 12, 1929, the basal metabolic rate had reached 1.1 per cent plus. The patient nevertheless was having occasional mild "spells of racing heart;" the dose of thyroid substance was reduced, therefore, to one-half grain twice daily. By September 21, the basal metabolic rate had dropped to 7.2 per cent minus.

The patient was seen at about monthly intervals from September, 1929 to October, 1930. Our effort has been directed to graduating the thyroid dosage, in a sort of compromise between the patient's subjective symptoms, the clinical findings, and the basal metabolic rate. This has not been as simple as one might at first suppose, since a dose sufficient to elevate and maintain her basal rate within normal limits was apt to precipitate an undue tachycardia. Seemingly, she had a low cardiac threshold for thyroid. However this may be, she has managed to avoid an attack of paroxysmal tachycardia during six months, while taking a dose of between one and one and one-half grains of thyroid substance daily. In an effort to determine whether the cardiac improvement was solely

ascribable to the thyroid administered, or partly to quinidine, the latter was omitted on 2 occasions for 2 months and 3 months respectively without any deleterious results.

Summary of Case One

A woman of fifty-one years who had suffered attacks of paroxysmal tachycardia for the previous 17 years, and between attacks was found to have a pulse rate of 50 and inverted T waves in all leads, presented characteristic clinical signs of adult myxedema, together with a basal metabolic rate of 31 per cent minus. Under thyroid therapy the myxedema disappeared not only, but a remarkable improvement occurred also in the cardiac condition which resulted in a transformation of inverted into upright T waves in the electrocardiogram. We are not prepared to go so far as to ascribe the attacks of paroxysmal tachycardia to myxedematous infiltration of the myocardium, particularly as the myxedematous changes, symptomatically at least, seemed to date back only a year. But we feel justified in assuming that the thyroid therapy was responsible for the cardiac improvement as evidenced by a marked diminution in the frequency of tachycardia attacks and by the striking changes in the electrocardiographic tracings.

COMMENTS FROM THE LITERATURE RELATIVE TO CARDIAC DISTURBANCES IN MYXEDEMA

The above narrative may now be compared with the experiences of others, though no pretense is made of an exhaustive search of the literature.

Zondek (1) in 1918 described 4 patients with myxedema showing a high degree of bilateral dilatation of the heart, bradycardia, and a sluggish movement of the heart borders when observed with the fluoroscope. These findings cleared entirely with thyroid extract.

Assman (2) and Meissner (3) each reported a case of myxedema with a similar story. Meissner described a second case in which the myxedema was complicated by chronic nephritis. There was marked clinical improvement with thyroid therapy, although the heart remained unchanged in size as demonstrated by the teleoroentgenograms.

Fahr (4) reported an extraordinary case of myxedema in a woman aged 46, in whom the heart was enormously dilated in all chambers with severe cardiac failure. Digitalis and complete rest seemed to have little effect on the heart. Thyroid extract, given in doses of 8 grains daily brought about remarkable improvement. The ascites and edema disappeared and the heart returned to normal size and shape. Fahr states "that cardiac failure is not only a prominent feature of all outspoken cases of myxedema, but a mild degree of heart failure with very slight dilatation of the heart is not at all infrequent in women patients past the age of 45, exhibiting slight but definite evidence of hypothyroidism."

Holzman (5) reported a case of myxedema of 18 years' duration, in which the heart was enormously dilated to the right and left, giving a transverse diameter of 19.7 cm. The size of the heart remained unchanged with thyroid therapy, although the symptoms of cardiac failure disappeared. In this instance the question is raised whether definite organic changes had taken place in the myocardium due to the long duration of the myxedema (18 years).

In contrast to the above reports, Willius and Haines (6) came to the conclusion after reviewing 162 of their cases of high grade myxedema,

that "none of the cases of heart failure or of organic cardiovascular disease could be justly attributed to myxedema . . . and that one is not justified in establishing a cardiac syndrome characteristic of myxedema." Ninety-one per cent of their cases gave no subjective or objective findings of organic cardiovascular disease. Of the remaining 9 per cent, whose hearts were definitely involved, only one showed any cardiac improvement (and this was moderate) from the administration of thyroid extract.

Means, White and Krantz (7) found, out of 48 patients with myxedema seen over a period of 12 years, only one in whom the heart approached the myxedema type of Fahr-Zondek. Christian (8) found no hearts of this type in 32 patients with myxedema, although 10 of these cases showed cardiac lesions.

Occasional reports are available of cardiac abnormalities in myxedematous patients which have vanished under the administration of thyroid substance, such as the case of Sturgis (9) with angina pectoris; Duden's (10) case of decompensation and hypertension; and Luten's (11) case of partial heart block.

On the other hand, there are reported instances of complete heart block in patients showing no evidence of myxedema which have likewise derived benefit from thyroid therapy. The case of Blackford and Willius (12) with Stokes-Adams syndrome and complete block falls in this category. Drake (13) was able to restore normal rhythm after complete block by administration of thyroid substance.

Of the various alterations in the electrocardiogram which may occur in myxedema, the most consistent finding is the absent or negative T wave. Thatcher and White (14) found in their series of 14 cases of myxedema a consistently low T wave in Lead II before treatment which disappeared with thyroid therapy. They concluded that a parallelism existed between the T waves and the basal metabolic rate. Zondek (1) and Fahr (4) observed the same changes. On the other hand, Willius and Haines (6) found normal electrocardiograms in 50 per cent of 49 cases. The other half showed negative T waves in various leads. Reid and Kenway (15), after studying 260 patients with low basal metabolic rates, concluded that there are no electrocardiograph changes characteristic of this condition. Five of their patients with myxedema, who had not been treated, showed "low T waves," and 4 of these had "low P waves." In a few cases notched and widened Q R S complexes of delayed intraventricular conduction disappeared under thyroid treatment (Fahr, 4).

The emphasis on the electrocardiographic abnormalities in myxedema as an indication of myocardial changes is somewhat minimized by the work of certain German investigators. Noble, Rosenblut and Samet (16) studied 11 children with myxedema. The electrocardiograms showed small, sometimes absent P and T waves; however, when they used needle electrodes applied to the chest wall the electrocardiograms were normal. They interpreted the changes in P and T waves as due to a changed resistance of the

skin in myxedema. Lueg (17) showed the same thing in another way; by using special electrodes he was able to change the electrocardiogram of a normal person into one with negative P and T waves, as in myxedema.

Although the finding of inverted T waves in one or two of the three leads has not been an uncommon occurrence, the presence of T inversion in all three leads and its conversion into upright waves as a consequence of thyroid administration would seem to be a rather rare experience. Duden (10) reports 1 such case; Willius and Haines (6), 3 cases.

Christian (8), Fishberg (18), Duden (10) and others have called attention to the premature arteriosclerosis which occasionally is found at autopsy of patients with myxedema. They suggest that this may be the underlying disease accounting for the cardiac disturbances. Wenckebach (19) comments on the common factor of water retention in myxedema and beri-beri and notes that swelling of the heart muscle interferes with its contractility. It is possible that by remedying the deficiency which occurs in these diseases (by supplying thyroid secretion or vitamin B, respectively), the water-logging of the heart muscle disappears, with consequent improvement.

Case II—Adult Myxedema Associated with Anemia of Primary Type
(Referring to Miss E. M.; U. C. O. P. D. No. 108462)

This patient was referred to our Endocrine Clinic, November 29, 1924; she came, originally, to the general Medical Clinic 6 months before, complaining of gastric pain which occurred sometimes before and sometimes after eating but which was temporarily relieved by food. The customary roentgenologic gastrointestinal findings were normal. A fractional gastric analysis, however, showed no free hydrochloric acid in the first 5 specimens (up to and including the one-hour fraction) and no subsequent reading above 8 degrees; total acidity varied from 12 to 25 degrees. This achlorhydria is of some interest in view of the subsequent findings of anemia and myxedema.

The patient was then treated by the Woman's Clinic and operated upon because of a right-sided ovarian cyst; the left tube was removed because of Neisserian infection which had been contracted a year before.

An item in the anamnesis, of severe sore throat and husky voice persisting for 6 weeks, at the age of 16, and diagnosed tuberculous, prompted a consultation with the Chest Clinic. No evidence of active tuberculosis was discovered; a roentgenogram disclosed cloudiness of the right apex. The patient had suffered from repeated respiratory infections, including 3 attacks of pneumonia between the ages of 20 and 24. A right pleural effusion with fever for 3 months complicated the first pneumonia.

The Woman's Clinic finally referred the patient to the Endocrine Clinic because of menorrhagia and a suspicion of hypothyroidism. At this time the patient was 30 years of age. Inquiry revealed that the patient was always cold, never perspired, was usually sleepy, that her hair had a tendency to fall out, and that these symptoms had gradually developed following an attack of typhoid fever 22 years before. Comparatively little was noted on physical examination; her voice was somewhat thick, the skin somewhat coarse, the pulse rate only 58. The complexion was somewhat pasty and the features slightly puffy. (See figure 4.) Because of her appearance and because of the symptoms noted above, hypothyroidism was considered probable, but we were hardly prepared for a basal metabolic rate of minus 33 per cent.

We were still further surprised by the following blood count: Hgb. 67 per cent; R. B. C., 2,736,000. It seemed reasonable to assume that this patient constituted one of the rather uncommon examples of myxedema associated with a primary type of anemia. In this connection it is appropriate to recall her achlorhydria. She was placed on thyroid therapy, at first, 6 grains of the desiccated substance daily; which was later increased to 8 grains daily.

Figure 4. Case II. Myxedema associated with anemia. Hemoglobin, 67 per cent; red blood cells, 2,736,000; basal metabolic rate, 33 per cent, minus. Note pasty complexion and slight puffiness of features.

Six weeks after beginning thyroid medication the patient's metabolism had been elevated to 6.52 per cent minus (January 30, 1925); the hemoglobin had risen to 75 per cent, and the red cells had increased to 4,080,000. Several white counts varied between 5,000 and 6,900. The lymphocyte count on three occasions was 43, 46 and 47 per cent.

In June, 1925, an electrocardiogram presented a rate of 85, and no abnormalities.

The patient by now was vastly improved, no longer cold and much more alert. She had also noticed that her hair was of finer texture (See figure 5). The very copious menstrual flow had been reduced to a normal quantity.

TABLE I

PATIENT, E. M., SHOWING RESPONSE OF BLOOD FORMATION TO THYROID ADMINISTRATION

Date	B. M. R.	Hgb.	R. B. C.	Medication
12/ 2/24	33.03%—	67% (S)	2,736,000	Started on thyroid extract, gr. vi daily, increased to gr. viii after two weeks.
1/30/25	6.52%—	75%	4,080,000	Thyroid gr. iii daily.
7/27/25	.....	75%	4,304,000	Thyroid gr. iii daily.
9/24/25	7.9 %—	75%	4,704,000	Thyroid gr. ii daily.
8/10/26	8.8 %+	....	.....	Thyroid gr. ii daily.
4/13/29	6.5 %+	78%	4,430,000	Thyroid gr. ii daily.
10/ 9/30	21.2 %—	78%	3,800,000	Thyroid gr. ii daily.

The thyroid dosage was gradually reduced to 2 grains daily. Subsequent basal rates and red cells and hemoglobin estimations may be found in the accompanying table. On one occasion some achromia and poikilocytosis was noted.

The patient faithfully continued the thyroid medication, and in the absence of any discomfort remained away from the clinic for a year and a half. When recently seen, although subjectively well, her basal rate had dropped to 21 per cent minus, and the red cell count had diminished to 3,800,000. A histamine test meal at this time showed no free hydrochloric acid. Her maintenance dose of thyroid will therefore have to be increased from 2 to probably 4 grains daily.

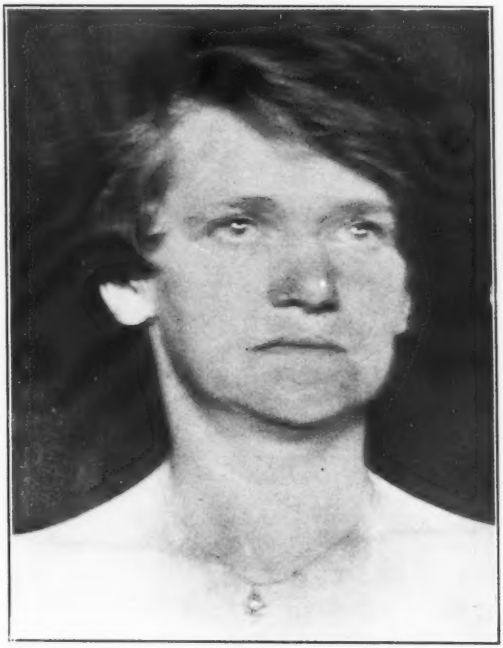


Figure 5. Case II. Myxedema associated with anemia. Compare with Figure 4. Note change in appearance due to thyroid therapy. Basal metabolic rate elevated from 33 per cent, minus, to 6.5 per cent, minus; hemoglobin elevated from 67 per cent to 75 per cent, and red blood cells from 2,736,000 to 4,704,000.

Summary of Case Two

A woman of thirty years who was always cold, never perspired, was usually sleepy, and had profuse menstrual bleeding, was found to have a basal metabolic rate of 33 per cent minus. In association with her myxedema was a blood picture suggesting primary anemia, hemoglobin 67 per cent (Sahli), red blood cells 2,736,000. Three fractional gastric analyses with several months intervening, showed complete achlorhydria on 2 occasions and no free hydrochloric acid in 5 out of 8 specimens in the third test, the highest value being 8 degrees. Under thyroid medication, the patient became symptomatically well, with a normal basal rate not only, but with a corresponding increase of the hemoglobin to 78 per cent and the red cells to 4,704,000. It seems reasonable to assume that the anemia, though of primary type, was secondary to the myxedema, and the improvement in the blood picture kept step with the restoration of the basal metabolic rate to normal.

COMMENTS FROM THE LITERATURE RELATIVE TO ANEMIA IN MYXEDEMA

Stone (20) states that Charcot (1881) was the first to record the observation that patients with myxedema might be "anemic to a high degree." Since then several writers have commented on this relationship (Kocher, 21; Pitfield, 22; Janney, 23; Boothby, 24; Minot, 25; Emery, 26; Kerppola, 27; Giffin and Bowler, 28; MacKenzie, 29; Stone, 20, and Means, Lerman and Castle, 30).

Anemia is not ordinarily a prominent feature in myxedema, but it may occur and be of severe grade. In some instances supplementary therapy with thyroid substance has been remarkably efficacious in restoring the hemoglobin and red count to normal levels. The anemia, when present, usually conforms to the secondary variety; but a high color index has been reported in several instances.

This peculiar combination of myxedema and pernicious anemia may be quite confusing. Ordinarily one of these two clinical pictures will be paramount and take precedence—that is to say, an unmistakable case of primary anemia may be complicated by a markedly low basal metabolic rate with suggestive signs of myxedema; or, vice versa, an unquestionable case of myxedema may be found to have a blood picture suggesting a primary anemia. Achlorhydria may occur in either instance. Still more rarely, one may encounter a patient showing both a pronounced myxedema and a severe pernicious anemia; there may be no dominance of one condition over the other in such a case, no interdependence, perhaps; indeed the two syndromes may be purely coincidental.

Recorded experience would seem to indicate that although anemia is more apt to occur when the basal rate is below 30 per cent minus, no constant parallelism obtains between the depth of the metabolic depression and the degree of anemia.

Even when the color index is high, the smear is more indicative of decreased blood formation than of increased blood destruction. Achromia may be present and likewise some anisocytosis. True microcytes and macrocytes are absent. Normoblasts have been found very rarely. Polychromasia is likewise infrequent.

The relative lymphocytosis first emphasized by Kocher (21) as characteristic of Graves' disease, is also seen occasionally in myxedema. The patient whose case has been reported above showed this differential preponderance.

If one ventures to lay the responsibility for the anemia at the door of hypothyroidism, one should expect to correct the blood picture by thyroid feeding. This has been successfully accomplished in many instances. Failure to accomplish this result might be anticipated in true pernicious anemia where the hypothyroidism is an accidental complication. In this connection, however, Briggs (31) points out, from his experience in 3 cases, that favorable results are unlikely if cord changes have occurred. He was firmly convinced that none of his 3 patients constituted examples

of true pernicious anemia; 2 of them, however, presented clinical evidence of combined system disease, and in both of them thyroid extract proved ineffective; the condition progressed and terminated fatally. From a prognostic standpoint, therefore, the presence of cord lesions would appear to be a matter of considerable importance.

It so happens that at this writing a patient is under observation whose hemoglobin is 37 per cent; red count, 1,100,000, and basal rate, 20 per cent minus. The patient was treated with thyroid rather than liver extract, with the hope that this therapy might influence the anemia. It was continued for slightly over a month, and although the basal rate was brought to normal, and remained there, repeated determinations of hemoglobin, red cells and reticulocytes failed to show even the slightest improvement. On the other hand, the patient's characteristic myxedematous symptoms, namely, marked puffiness of the tissues and scaly dryness of the skin, responded promptly to thyroid therapy. Liver extract was then added with typical rise in reticulocytes, and within 3 weeks the hemoglobin rose from 35 per cent to 60 per cent. This case corresponds to a series of 5 recently published by Means, Lerman and Castle (30), where there appeared to be nothing more than an "accidental association" of pernicious anemia and myxedema, each disease responding to its specific treatment.*

Case III—Adult Myxedema Associated with Menorrhagia
(Referring to Mrs. M. R.; U. C. O. P. D. No. 20346)

A woman of forty-one years of age, who was born and lived in Italy until 13 years of age, had been reporting off and on for several years to the Woman's Clinic of the University of California Medical School. She had never suffered from any noteworthy diseases. Operations included dilatation and curettage, cervical amputation, anterior and posterior colporrhaphy and umbilical herniotomy in July, 1918. She was 37 years old at that time.

She had reared 7 children; the only item of endocrine significance is that one son was not growing properly and weighed only 32 pounds at the age of 6.

She had been disturbed for some years by profuse menstrual periods occasionally amounting to actual hemorrhage. At one time a fibroid uterus was suspected as responsible for the menorrhagia; hysterectomy was advised, but fortunately, in view of subsequent developments, the patient refused. At another time the insertion of radium was recommended, but again the patient demurred. Stypticin and ergotin were resorted to without benefit. Finally, after repeated examinations, the pelvis was exonerated as a mechanical cause for her profuse menstrual flow. About this time some one noted marked puffiness of the features and referred the patient to the Medical Clinic with a suspicion of either myxedema or nephritis. As is commonly the case, the latter possibility was granted precedence, but, beyond a faint trace of albumin, extensive renal function studies (including phenolsulphonephthalein test, blood urea, non-protein nitrogen, creatinin and uric acid, and an Addis test) revealed no evidence of nephritis.

She came to the Endocrine Clinic in June, 1922. The puffiness of the face, more particularly the eyelids, the swollen tongue, hands and feet, the dry, rough skin, the apple blossom cheeks (malar flush on a pasty yellow background), the low-pitched voice, bradycardia of 54, and the subjective feeling of coldness—formed a characteristic combination of symptoms and signs necessitating the clinical diagnosis of myxedema (See figures 6 and 7). It was, furthermore, reasonable to assume that the menorrhagia was consequent to this thyroid insufficiency. A basal metabolic rate of 41.6 per cent minus (June 19, 1922) abundantly confirmed the clinical diagnosis.

*This case is to be reported in detail in a separate paper by Doctors S. R. Mettier and E. M. Anderson.

Figures 6 and 7. Case III. Myxedema associated with menorrhagia. Note puffiness of face—more particularly the eyelids. Basal metabolic rate, 41.6 per cent, minus.

The patient was at once placed on thyroxin, orally, and this preparation was continued for 3 months. During this time the following improvement occurred: elevation of the basal rate to normal (0. on September 1, 1922); disappearance of the myxedematous puffiness (See figure 8); progressive "thawing out" with a pleasant feeling of warmth; and regulation of the menstrual cycle to 28 days, whereas prior to treatment periods occurred 3 times every 2 months. The flow, however, remained extremely copious.

It was then decided to utilize ordinary thyroid substance (Armour's) instead of thyroxin, the dose varying from three to eight grains daily. Under this regime the menorrhagia disappeared and the menstrual periods continued regular and became quite normal.

The dose of thyroid substance was gradually reduced to 2 grains of the dessicated material daily. The patient reported every few months from the onset of treatment in June, 1922, to the present (January, 1931). During these many years of observation, the patient, on at least three occasions, disobeyed our admonition to be faithful in never missing her daily dose of thyroid substance. Invariably, when she regarded herself as cured and ceased taking thyroid (once for 2 months and again for as long as 5 months) the myxedematous phenomena recurred; menstruation became more frequent; and the flow remarkably excessive. Admission of guilt and resumption of treatment was each time followed by prompt relief of symptoms.

On July 27, 1928, at the age of 47, and when the patient was menstruating, her husband suddenly died. The shock of his death seemingly produced an emotional menopause. Since that time the patient has menstruated 3 times only. the last period occurring in February, 1930, a year after the previous period.

It was disconcerting, on the two or three occasions noted above when the patient voluntarily discontinued her thyroid, that, although the myxedematous symptomatology recurred, a check of her basal metabolism proved to be normal. The writers suspected that the determinations were incorrect. though no error could be detected. Finally, however, October 30, 1930, after offending again by omitting thyroid for a month, her basal rate had sunk to minus 30 per cent.

Summary of Case Three

A woman of forty-one years who had reared 7 children suffered from recurrent menorrhagia which had persisted despite stypticin and ergotin. Fortunately, hysterectomy and radium implantations had been refused. She was found to be characteristically myxedematous, with a basal of 41.6 per cent minus. Under thyroid therapy the myxedematous puffiness and intolerance for cold vanished not only, but her menstrual cycles which had recurred 3 times every 2 months, and which had been very profuse, became regularized to a normal 28-day periodicity, and the flow was diminished to a normal amount. On several occa-

Figure 8. Case III. Myxedema associated with menorrhagia. Compare with Figures 6 and 7. Note striking alteration in patient's features. Basal metabolic rate elevated from 41.7 per cent. minus, to zero. Frequent periods with very copious flow changed to normal intervals with normal flow by thyroid therapy.

sions, despite our admonitions, the patient discontinued thyroid therapy and invariably myxedema and menorrhagia recurred. Resumption of therapy produced prompt relief each time.

This narrative is presented in order to emphasize the importance of recognizing hypothyroidism as a cause of menorrhagia. In the absence of unquestionable polyps (and perhaps even in their presence, before radical operative measures are undertaken) determination of the basal metabolism is an advisable procedure.

COMMENTS FROM THE LITERATURE RELATIVE TO MENORRHAGIA IN MYXEDEMA

Menorrhagia is quite characteristic of depressed thyroid activity in adult life. There is general agreement on this point. However, Kern (32) (loc. cit.) remarks that although "myxedema is usually accompanied by menorrhagia, amenorrhea may also occur." Verco (33), Crotti (34)

and Bell (35) make similar statements. Gardner-Hill and Smith (36) report that myxedema occurring before the natural menopause is associated with menorrhagia in 78 per cent of cases. Falta (37) writes the following: "In severe cases of myxedema women develop irregularities of menstruation, either the menses cease, or there occur profuse floodings. Starr, Kirk, Shotten, and after them many others, have testified to the occurrence of menorrhagia." The writers have had the same experience as Berkeley (38), who remarks, "There are some cases of menorrhagia that seem to be much benefited by thyroid extract." Finally, McCarrison (39) may be quoted: "When the thyroid defect arises later in life menorrhagia is the rule. The menstrual period is prolonged and the intervening periods shortened. Hertoghe considers that the infiltration of uterine mucosa and musculature, as well as the diminished coagulability of the blood, is responsible for the profuse menstrual discharge which is so characteristic of the condition." Isbister (40), in a paper entitled "Menorrhagia with an Apparently Normal Pelvis," refers to a report of Sehrt "that of 85 patients with functional menorrhagia without any pelvic lesions, 69 per cent suffered from hypothyroidism."

SUMMARY

1. A case of adult myxedema with attacks of paroxysmal tachycardia is reported. Under thyroid medication the inversion of T waves in all 3 leads was converted into the normal upright form.

2. A case of adult myxedema with an anemia having a high color index is reported. Under thyroid medication the blood picture became normal.

3. A case of adult myxedema with severe menorrhagia is reported. Menstruation both as regards periodicity and amount of flow was restored to normal under thyroid therapy.

4. These 3 cases are placed on record in order to emphasize the importance of recognizing that marked anemia, severe menorrhagia, and striking cardiac irregularities may occasionally be due to hypothyroidism, and if this underlying factor be discovered, appropriate thyroid medication will, ordinarily, produce pronounced improvement.

BIBLIOGRAPHY

Cardiac Disturbances in Myxedema

1. Zondek, H.: Das Myxödemherz. München. med. Wchnschr. **65**: 1180. 1918.
2. Assmann, H.: Das Myxödemherz. München. med. Wchnschr. **66**: 9. 1919.
3. Meissner, R.: Zur Klinik des Myxödemherzens. München. med. Wchnschr. **67**: 1316. 1920.
4. Fahr, G.: Myxedema heart. J. A. M. A. **84**: 345. 1925.
5. Holzman, J. E.: Myxedema heart. Am. Heart J. **4**: 351. 1929.
6. Willius, F. A. and S. F. Haines: Status of heart in myxedema. Am. Heart J. **1**: 67. 1925.
7. Means, J. H., P. D. White and C. T. Krantz: Observations on the heart in myxedema. Boston M. & S. J. **195**: 455. 1926.
8. Christian, H. A.: The heart and its management in myxedema. Rhode Island M. J. **8**: 109. 1925.
9. Sturgis, C. C.: Angina pectoris as a complication in myxedema and exophthalmic goiter. Boston M. & S. J. **195**: 351. 1925.

10. Duden, C.: Myxedema with cardiac decompensation and hypertension which disappeared under thyroid medication. J. Missouri State Med. Assn. **26:** 25. 1929.
11. Luten, D.: Myxedema with partial heart block and severe anemia, both of which disappeared under thyroid therapy. J. Missouri State Med. Assn. **26:** 73. 1929.
12. Blackford, J. M. and F. A. Willius: Chronic heart block. Am. J. M. Sc. **154:** 585. 1917.
13. Drake, E. H.: A case of complete heart block with interesting reaction to drugs. Am. Heart J. **31:** 560. 1928.
14. Thatcher, C. and P. D. White: The electrocardiogram in myxedema. Am. J. M. Sc. **171:** 61. 1926.
15. Reid, W. D. and F. L. Kenway: Electrocardiographic signs associated with low basal metabolism. This Journal, **13:** 191. 1929.
16. Noble, E., A. Rosenbluth and B. Samet: Das Elektrokardiogramm des kindlichen Myxödems. Ztschr. f. d. ges. exper. Med. **43:** 332. 1924.
17. Lueg, W.: Haut und Elektrokardiogramm. Arch. f. d. ges. Physiol. **212:** 649. 1926.
18. Fishberg, A. M.: Arteriosclerosis in thyroid deficiency. J. A. M. A. **82:** 463. 1924.
19. Wenckeback, K. F.: Heart circulation in a tropical avitaminosis. Lancet **2:** 265. 1928.

Anemia in Myxedema

20. Stone, C. T.: The occurrence of anemia in myxedema. Ann. Int. Med. **2:** 215. 1928.
21. Kocher, T. L.: Das Blutbild bei Cachexia thyreopriva. Arch. f. Klin. Chir. **99:** 280. 1912.
22. Pitfield, R. L.: Myxedema and the nervous system. Am. J. M. Sc. **151:** 409. 1916.
23. Janney, N. W. and H. E. Henderson: Concerning the diagnosis and treatment of hypothyroidism. Arch. Int. Med. **26:** 297. 1920.
24. Boothby, W. M.: Oxford System of Medicine, Vol. III, Part II, p. 946, Oxford University Press, New York, 1921.
25. Minot, G. L.: Two curable cases of anemia. M. Clin. North America, **4: 2:** 1733. 1921.
26. Emery, E. S.: The blood in myxedema. Am. J. M. Sc. **165:** 577. 1923.
27. Kerppola, W.: Finska läk-sällsk. handl. **65:** 330. 1923.
28. Giffin, H. Z. and J. P. Bowler: Diseases which may be associated with pernicious anemia. Minnesota Med. **6:** 13. 1923.
29. MacKenzie, G. M.: Anemia in hypothyroidism. J. A. M. A. **86:** 462. 1926.
30. Means, J. H., J. Lerman and W. R. Castle: The coexistence of myxedema and pernicious anemia. Tr. A. Am. Physicians, **45:** 363. 1930.
31. Briggs, L. H.: Personal communication.

Menorrhagia in Myxedema

32. Kern, M.: The thyroid gland and menstrual disorders. Clin. Med. & Surg. **35:** 473. 1928.
33. Verco, J.: Myxedema. M. J. Australia, **2:** 2. 1925.
34. Crotti, A.: Thyroid and Thymus. 1926. Lea and Febiger, Phila. and New York. P. 116.
35. Bell, W. Blair: The Sex Complex. 1921. Wm. Wood and Co., New York.
36. Gardner-Hill, H. and J. F. Smith: The influence of disease of thyroid on menstruation. J. Obstet. & Gynaec. Brit. Emp. **34:** 701. 1927.
37. Falta-Myers: Endocrine Diseases. 1923. P. Blakiston Son and Co. P. 92.
38. Berkeley, W. N.: Principles and Practice of Endocrine Medicine. 1926. Lea and Febiger, Phila. and New York. P. 116.
39. McCarrison, R.: The Thyroid Gland. 1917. Wm. Wood and Co., New York. P. 221 and P. 186.
40. Isbister, J. L. T.: Menorrhagia with an apparently normal pelvis. M. J. Australia, **2:** 165. 1928.

AN ADRENAL CORTEX EXTRACT: ITS DEVELOPMENT AND EXPERIMENTAL AND CLINICAL USE*

RAYMUND L. ZWEMER

From the Department of Anatomy
College of Physicians and Surgeons, Columbia University
NEW YORK

The adrenal or suprarenal gland consists of cortex and medulla, which are embryologically of separate origin, yet are found in intimate relationship in the warm-blooded animals. It has been found that complete removal of these glands is always fatal. Accessory tissue, which may prevent death, is often present in laboratory animals such as the rat, but is quite rare in normal cats and dogs.

The cortex is the portion of the complex necessary for life and is responsible for most, if not all, of the symptoms occurring after adrenal removal, since complete demedullation of one gland and removal of the other fails to produce symptoms of adrenal insufficiency. The cortical remnant maintains life, but its removal is fatal. Both dogs (1) and cats (2), (3), have been subjected to these operations. They show that the medullary tissue in the adrenal complex is not essential, although the medulla has an important function as a strengthener of the sympathetic nervous system and may even act in the absence of sympathetic connections (4). Adrenin's action, it must be remembered, occurs rapidly and disappears within a very short time. Its place in the organism after adrenalectomy may possibly be taken over by the sympathetic nervous system and "Sympathin" (5), (6).

Even blood sugar is not entirely dependent upon the presence of the adrenal medulla. Animals maintained by a surgically produced cortical remnant had a blood sugar level within the normal range (7). Removal of this cortex was followed by a decrease in blood sugar.

In an attempt to elucidate the normal function of the adrenals from a study of the pathologic changes occurring in the organism after their removal, gross and microscopic examinations of the organs of cats suffering from uncomplicated adrenal insufficiency were made. The absence of complications is stressed because of the well known susceptibility of all adrenalectomized animals to intercurrent infections. In addition to diminished subcutaneous fat, marked dehydration, and moderate congestion of the viscera, there were histologic changes in the kidneys, lymph nodes, thyroids and testes (8).

In the kidney there was fatty degeneration of the tubules. The cells lining the convoluted tubules were so distended with large and small

*Read before the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 9, 1931.

vacuoles that the lumina were no longer visible. Even the cells in the loops of Henle and the collecting tubules contained vacuoles in the cells and fatty casts in their lumina. Differential fat staining showed the presence of both neutral fats and other lipids. The lesions are quite definite and the fat is much more abundant than is normal in the kidney of the cat.

The lymph nodes showed enlargement, their germinal centers were active and the surrounding sinuses filled with mature lymphocytes. This lymphoid hyperplasia can be correlated with a relative and absolute increase in lymphocytes in the circulating blood. The change in the differential count progressed concommitantly with other symptoms of adrenal insufficiency (9).

TABLE I
LEUCOCYTE CHANGES AFTER ADRENAL REMOVAL
(Zwemer & Lyons)

	Total White Blood Cell Counts	Differential Count				
		Neutrophiles	Eosinophiles	Monocytes	Lymphocytes	Other Cells
Normal	5,000–15,000	66.8	6.1	3.6	23.3	0.2
Uncomplicated adrenal insufficiency	5,000–14,000	40.9	4.6	3.7	49.3	1.5
Presence of infection	14,000–69,000	83.1	1.2	4.9	10.7	0.1

The thyroid was in what is known as the "resting state." This agrees with the lowered basal metabolism found in adrenal insufficiency.

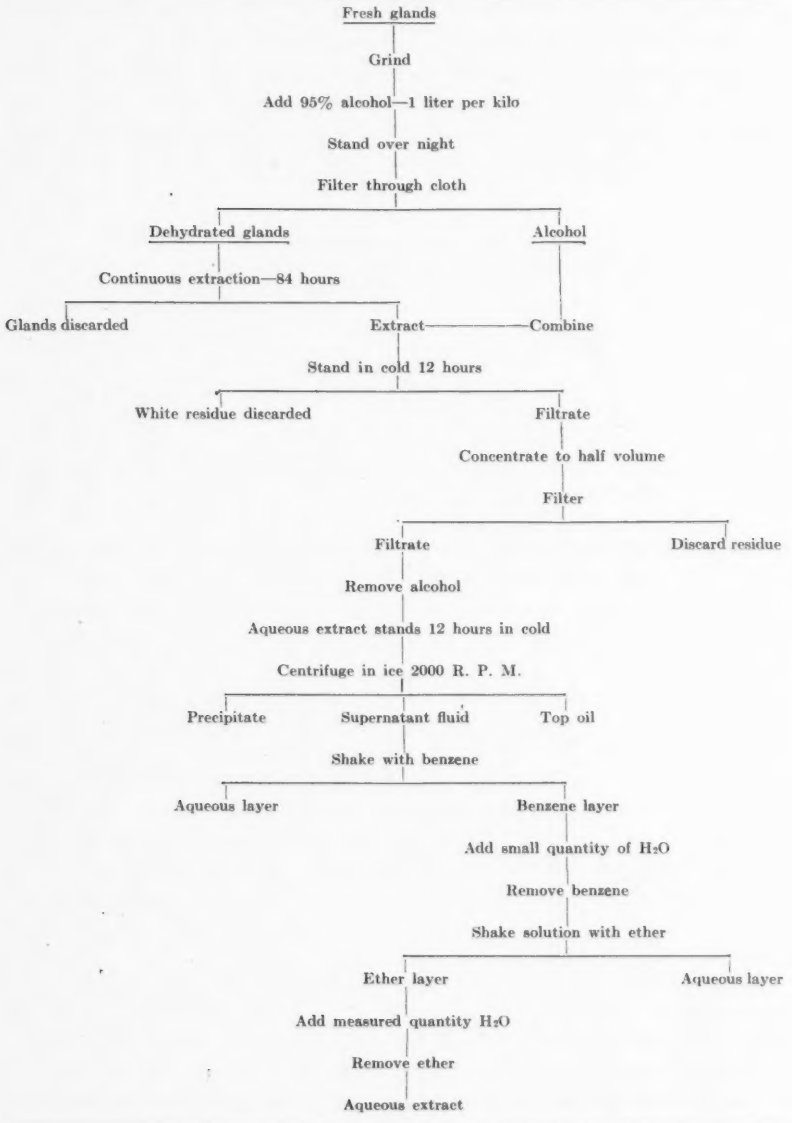
The testes showed an increase in the number and size of the interstitial cells. A gonad-adrenal interaction has long been favored from a clinical aspect (10).

Substitution for the adrenal has been attempted by a great many investigators, but even autoplastic transplantations have never been very successful. Only Jaffe (11) and Wyman (12) have reported favorable results in guinea pigs and rats. Due to the success of P. E. Smith (13) with daily anterior pituitary implants, we attempted daily implants of sheep adrenal cortex (14). These had an ameliorating effect on the general symptoms of adrenal insufficiency but their toxic local effects prevented their use over any considerable period of time. We finally decided to make extracts of the adrenal cortex to see whether a hormone free from toxic by-products could be obtained.

The adrenal cortex extract was prepared by Zwemer, Agate and Schroeder (15) and Table 2 gives a synopsis of the method reported by them. It is essentially a thorough extraction of beef adrenals with ethyl alcohol, followed by physical and chemical removal of unwanted products. The potency of the various batches and fractions of extract were determined by their effect on the clinical and chemical symptoms of adrenalectomized cats, and also by their effect on normal cats and rabbits.

TABLE II

PREPARATION OF ADRENAL CORTEX EXTRACT
(Zwemer, Agate & Schroeder)



Blood chemical changes in adrenal insufficiency have been described by many investigators (16, 17, 18, 19, 20, 21). In a search for a criterion of the degree of adrenal insufficiency present in an animal at a given time, Zwemer and Sullivan (22) made a chemical study of the blood of cats at varying periods after adrenal removal. We found that the average CO_2 capacity dropped from 37 to 23 volumes per cent. The blood chlorides (as NaCl) fell from 625 mgm. to 575 mgm. per 100 cc. and the average blood sugar decreased from 109 mgm. to 66 mgm. per 100 cc.; on the other hand the phosphates rose from 5.6 mgm. to 8.2 mgm. per cent, and the average non-protein nitrogen increased from 43 mgm. to 88 mgm. per hundred cc. of blood. These averages were obtained from determinations on more than twenty cats and include figures from all stages of insufficiency. The figures obtained a few hours before death are even more striking.

Much to our surprise we found that after removal of only one adrenal the average trend of these five blood constituents was in the direction of adrenal insufficiency, although the outward appearance and actions of the cats were normal.

The clinical symptoms of adrenal insufficiency include asthenia, anorexia, dehydration with loss of tissue turgor, decreased or stagnated peripheral circulation and early coldness of the extremities, although the rectal temperature may be high. The body temperature is sub-normal for some time before death. An increase in the red blood cell count and the hematocrit reading when contrasted with the lowered blood volume indicates a lowering of the plasma volume along with the tissue dehydration. This decrease in plasma volume would accentuate the lowered carbon-dioxide capacity of the blood as a whole, since each unit volume of plasma has a decreased carbon-dioxide capacity.

Our successful extracts brought about a rapid improvement in both the clinical and chemical picture of adrenal insufficiency. Animals unable to stand up, walk or eat were walking around and eating food within an hour after injection of the extract. Their blood picture began to return to normal in a very short time.

Normal animals reacted to injections of our adrenal cortex extract by an increase in their carbon-dioxide capacity and blood sugar. Individual variations from the normal blood chlorides and non-protein nitrogen were also favorably affected. The use of normal cats and rabbits permitted more rapid and frequent testing of the extracts and required much smaller amounts for injection. Over five hundred blood-chemical findings in treated and untreated, normal and adrenalectomized animals will be dealt with in detail in a paper to appear shortly by Zwemer and Sullivan.

Doctors MacLean and Sullivan have been gathering data on the blood-chemical picture in intestinal intoxication in more than two hundred babies. In the severe cases the findings were similar to those of experimental adrenal insufficiency (23). The clinical picture was also like that of adrenal insufficiency, although there were always the complicating factors of inter-

current infections and the transient effects of the various treatments instituted (24). It was decided that a few of the more severe cases, which had failed to improve with the usual treatments, might benefit by an injection of our adrenal cortex extract.

Even very small amounts of the extract, injected intramuscularly, brought about a favorable change within twenty-four hours. Most of the babies so treated were discharged from the hospital cured. The death of a few was due to causes other than intestinal intoxication, but, the symptoms of the latter syndrome disappeared in all cases but one. Our failure in this instance could be attributed to a loss of potency of the extract used, as shown by its inactivity when injected into test animals.

The blood-chemical test for adrenal cortex extract is not yet quantitative but it is qualitative. A single injection of the potent extract will give a positive test in normal cats and rabbits, adrenalectomized cats and in babies with intestinal intoxication. Non-potent fractions and various control substances are consistently negative for all subjects.

The presence of adrenin or of an adrenin-like contaminant in the extract can be detected by an initial drop in the carbon-dioxide capacity of the serum and a rapid increase in blood sugar.

A lack of uniformity in the reaction of the different animals to the same amount of extract per kilogram of body weight was at first confusing. As the number of cases increased, however, it was discovered that animals with a low carbon-dioxide capacity reacted to a greater degree than those with an initial high value. When treated statistically it was found that the figures from all normal test animals were grouped around a common level which was about twenty-six per cent higher than the average carbon-dioxide capacity before the injections were given. Changes in the other blood constituents of normal cats will be reported in the near future.

The researches reported in the above paper cover the work done by the author and his collaborators in six institutions during the past eight years. He takes great pleasure in acknowledging his indebtedness to all who have contributed to this study. The bibliography includes all but the most recent, unpublished separate contributions.

SUMMARY

The clinical, blood cell, blood chemical and pathologic changes occurring in over two hundred cases of experimental adrenal insufficiency have been studied.

We believe that most if not all of these changes are due to an absence of the adrenal cortex, since the presence of a surgically produced cortical gland in the animal prevents the above mentioned changes.

An adrenal cortex extract has been prepared, which when injected into animals brings about a reversal of the effects produced by adrenalectomy.

Babies suffering from severe intestinal intoxication, which resembles adrenal insufficiency, have been favorably affected by injections of the adrenal cortex extract.

Blood-chemical changes in normal animals are indicative of the potency and purity of the extract and show whether adrenin or adrenin-like contaminants are present.

BIBLIOGRAPHY

1. Houssay, B. A. and J. T. Lewis: Am. J. Physiol. **64**: 512. 1923.
2. Zwemer, R. L.: Anat. Rec. **29**: 103. 1924.
3. Zwemer, R. L.: Am. J. Physiol. **79**: 641. 1927.
4. Zwemer, R. L. and H. F. Newton: Am. J. Physiol. **85**: 507. 1928.
5. Newton, H. F., R. L. Zwemer and W. B. Cannon: Am. J. Physiol. **96**: 377. 1931.
6. Cannon, W. B. and Z. M. Bacq: Am. J. Physiol. **96**: 392. 1931.
7. Zwemer, R. L., J. M. Smith and M. Shirley: Anat. Rec. **45**: 250. 1930.
8. MacMahon, H. E. and R. L. Zwemer: Am. J. Path. **5**: 491. 1929.
9. Zwemer, R. L. and C. Lyons: Am. J. Physiol. **86**: 545. 1928.
10. Glynn, E. E.: Quart. J. Med., Oxford, **5**: 157. 1912.
11. Jaffe, H. L.: Am. J. Path. **2**: 421. 1926.
12. Wyman, L. C.: Am. J. Physiol. **87**: 29. 1928.
13. Smith, P. E.: J. A. M. A. **88**: 158. 1927.
14. Zwemer, R. L. and P. Lerner: "Daily transplants of sheep adrenal cortex." (Unpublished.)
15. Zwemer, R. L., F. J. Agate Jr. and H. A. Schroeder: Proc. Soc. Exper. Biol. & Med. **28**: 721. 1931.
16. Lucas, G. H. W.: Am. J. Physiol. **77**: 141. 1926.
17. Rogoff, J. M. and G. N. Stewart: Am. J. Physiol. **78**: 711. 1926.
18. Baumann, E. J. and S. Kurland: J. Biol. Chem **71**: 281 1926-27.
19. Swingle, W. W.: Am. J. Physiol. **79**: 666. 1927.
20. Hartman, F. A., C. G. MacArthur, F. D. Gunn, W. E. Hartman and J. J. MacDonald: Am. J. Physiol. **81**: 244. 1927.
21. Yonkman, F. F.: Am. J. Physiol. **86**: 471. 1928.
22. Zwemer, R. L. and R. C. Sullivan: Proc. Soc. Exper. Biol. & Med. **28**: 723. 1931.
23. Sullivan, R. C., A. B. MacLean and R. L. Zwemer: N. Y. Acad. Med. May 14, 1931. Pediatric Section.
24. MacLean, A. B., R. C. Sullivan and R. L. Zwemer: N. Y. Acad. Med. May 14, 1931. Pediatric Section.

STUDIES ON FAT METABOLISM. III. LIPEMIA INDUCED BY INTRAVENOUS FAT ADMINISTRATION*

HUGO R. RONY, M.D., AND BERNARD MORTIMER, Ph.D.
From the Departments of Medicine and Physiology, Northwestern University Medical School
CHICAGO

The study of the removal of fat from the blood after inducing lipemia by oral administration is hindered by the fact that lipemia in these cases is a resultant of two processes: passage of fat into the blood and from the blood into the tissues. The passage of fat into the blood is a complicated process depending upon the motility of the bowel, and upon the rate of digestion and absorption of the fat which in turn depends upon nervous and hormonal factors controlling bile secretion, bile evacuation, pancreatic secretion, etc. Therefore conclusions as to the rate of removal of fat from the blood must be drawn in such experiments with caution (1). In investigating the effects of various physiological factors on alimentary lipemia it is difficult to insure standard conditions under which the passage of fat into the blood is sufficiently uniform. To eliminate this difficulty and to obtain more uniform standards by which to evaluate the effect of influencing factors, in these experiments, we introduced the fat into the blood stream directly.

A study of the literature shows that some similar experiments have been performed. Bondi and Neumann (2) injected colloidal suspensions of fat intravenously and examined the blood with the ultramicroscope. They found that the fat particles disappear from the blood within one-half hour. Schott (3) injected egg yolk intravenously and with the same method of examination got the same result. Raper (4) injected olive oil emulsified with casein into dogs and cats and found 25-60 per cent of the fat in the liver after 3-4 hours. The blood fat was not examined. Bloor (5) used egg yolk diluted with equal volume of saline solution. He injected 10 grams of fat in 8 minutes into the jugular vein of a dog and found that after 18 minutes practically all of the injected fat was still present in the blood. The fat then disappeared, at first rapidly, then at a slower rate, the previous level being reached after 7 hours. A similar experiment was performed on the same dog after it was fasted for 8 days, with the result that removal of the injected fat was more rapid than before. No appreciable change in the blood fat was observed as the result of injections into the blood stream of olive oil emulsified with casein (made according to the directions of Raper, the size of the particles being two to five microns) or of colloidal suspensions of fat (containing nothing but fat, salt and water, the size of the particles being 1 micron), although

*Read before the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 8, 1931.

enough fat was injected to raise the percentage of fat from 50 to 100 per cent. Bloor estimated the fat by his nephelometric method. Saxl and Donath (6) injected a 20 per cent commercial oil emulsion (composition not stated) with particles "not larger than hematoconia." The fat content of the blood was estimated by the use of the ultramicroscope. Sections of the liver, lung, kidney and spleen were examined after having been stained with Sudan. They found that the fat particles disappeared from the blood in 10-15 minutes and that the reticulo-endothelial cells of the above-named organs were filled with fat droplets after this time. Thyroidin promotes the removal of the fat particles from the blood and the ladening of these organs with fat, while pituitrin and insulin inhibit it. Markowitz and Mann (7) injected an emulsion of fat prepared by emulsifying olive oil with blood serum and sodium glycocholate, containing particles not larger than 4 microns. Ten minutes after the injection the blood from the right and left heart contained 2.86 and 2.55 per cent fat respectively. The fat content of the lung rose from 2.8 to 18 per cent and that of the liver from 3.5 to 19 per cent. When citrated chyle obtained from the thoracic duct of a dog previously fed with fat was injected into the jugular vein the fat content of the lung showed no change, and the blood fat returned to normal in 40 minutes, although the chyle contained 2.9 gm. fat.

Experimental data based on the use of the ultramicroscope as a method for estimating lipemia are wholly unreliable, as will be shown later. The use of fat emulsions containing particles larger than 4 microns to induce lipemia is likewise open to serious objection. Finally the introduction into the blood stream of concentrated fat emulsions at too great a rate does not represent physiological conditions. In our experiments we tried to avoid these objectionable features.

EXPERIMENTAL PART

Choice of emulsion: An examination of various high grade commercial emulsions of fat revealed that in all the particles were of such size as to preclude the possibility of their use for our purpose. Several preparations which we made were likewise unsatisfactory. This led us to the adoption of egg yolk which diluted with saline solution yields an emulsion with particles ranging in size from one-half to 2 microns. In this way the possibility of mechanical interference with the circulation of the emulsion in the blood stream is avoided. Also, while colloidal fat emulsions, as prepared by Raper and by Bloor, disappear from the blood stream very quickly, intravenously introduced egg yolk emulsion stays in the blood for a prolonged period of time permitting the study of any factors which may possibly influence the removal of the fat.

Method: For the preparation of the emulsion one-day old eggs were used. The white was carefully separated from the yolk, which was then washed with sterile saline solution several times in a sterilized vessel. The yolk membrane was then removed and the remainder weighed. It was then ground thoroughly with sterile saline so as to contain 1.5 grams of yolk per kilogram body weight of the animal. For the smaller dogs 120 cc. of emulsion was made up and for the larger dogs 180 cc. The emulsion was injected at a constant rate of 2 cc. or 3 cc. per minute for 1 hour by the Woodyatt pump. The fat concentration of these emulsions varied from 4 to 5.5 per cent thus approximating closely physiological conditions inasmuch as after fat meals at the height of absorption fat pours at a similar rate through the thoracic duct into the blood. The emulsion warmed to body temperature was introduced into the saphenous vein, the needle

being taped to the leg. The dogs were trained to lie quietly on the table and no hypnotics nor anesthesias were used. The injections were tolerated well; frequently evacuation of the bowel occurred, and occasionally vomiting. Injections repeated within a week were tolerated equally well, but if more than 7 days had elapsed, protein or anaphylactic shock was experienced. For this reason not more than two or three experiments were carried out on the same dog within the period of 7 days, the dog not being used thereafter. It was noticed that these dogs ate ravenously when fed after the experiment, even when the blood fat was as high as 2 per cent.

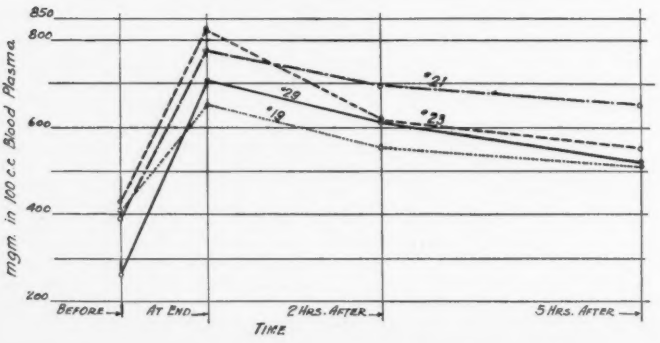
For the chemical examination of the blood, specimens were drawn from a vein of the foreleg just before the injection was started, at the end of the injection, 2 hours after the end, and 5 hours after the end. The plasma of the citrated blood was analyzed for total fatty acids by Bloor's oxidation-titration method (8). Visible lipemia—the formation of cream on the centrifugalized plasma—was not observed even though in some experiments the fat content was more than 2 per cent. However, the plasma obtained after the injection was more or less opalescent and slightly yellowish. Lecithin was determined by Bloor's latest method (9).

Normal removal from the blood of injected fat: The dogs used in these experiments were starved for 24 hours. In 3 of the 4 experiments the plasma volumes were also determined to observe the degree of a possible dilution or concentration following the injection. In dog No. 19 the values were 69 per cent at the beginning, 65 per cent at the end, 66 per cent after 2 hours, and 69 per cent after 5 hours. In dog No. 21 the corresponding values were 65 per cent, 68 per cent, and 68 per cent. In dog No. 23 the values were 63 per cent, 60 per cent, 62 per cent, and 61 per cent. These changes hardly exceed normal variations and limits of error. The results of the fat analyses are shown in Table 1.

TABLE 1

LIPEMIA INDUCED BY INTRAVENOUS EGG YOLK ADMINISTRATION (STANDARD CURVES FOR COMPARISON)

DOG NO	TOTAL FATTY ACIDS mgm. in 100 cc Blood Plasma				
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	5 HOURS AFTER END	20 HOURS AFTER END
19	415	659	565	515	
21	382	775	692	648	
23	435	831	623	554	457
29	258	702	619	522	



INTERPRETATION OF RESULTS

It is seen from Table I that there is a sharp rise in the total fatty acid content of the plasma at the end of the injection, followed by a slow disappearance in the succeeding hours. After 5 hours the total fatty acids are still far above the initial level which is not quite reached even after 20 hours. It is also seen that the rate of removal is quite uniform in all cases.

COMPARISON OF ALIMENTARY AND INTRAVENOUSLY INDUCED LIPEMIA

The lipemia following fat feeding by mouth is characterized by a comparatively slight increase in the blood fat with a maximum after 5 hours and a rapid decline thereafter (1). It is true that in the alimentary feeding the fat is being poured into the blood for a long period of time due to its slow absorption from the bowel. However, it has been shown by Munk and Rosenstein (10) in an experiment carried out on an 18 year old girl with a lymph fistula that very little fat reaches the blood stream through the thoracic duct in the first 3 hours and after the 10th hour following the ingestion of fat and that the bulk of the absorbed fat is carried between the 4-9 hours, the fat concentration in the chyle being 3.24 per cent in the 4th hour, 4.3 per cent in the 5th hour, and 3.4 per cent in the 6th hour. These data indicate that after fat feeding fat is reaching the blood stream at a rate and in concentrations comparable with the rate and concentration used in our intravenous injections of egg yolk emulsions. Yet the lipemia after fat feeding is comparatively very slight, or is entirely missing (1), and in some cases (obese persons) the fat feeding may be followed by an actual decrease in the total fatty acids (11).

This contrasting behavior necessitates an analysis of the factors different in the two kinds of fat administration to determine the possible cause of the dissimilar rates of removal of the fats.

In the first place the egg yolk administered intravenously must be compared with the fat carried by the thoracic duct into the blood stream in alimentary lipemia. One-third of the total fatty acids of egg yolk is lecithin which might not have been present in appreciable amounts in the chyle after fat feeding. Also the physical state of dispersion of the fats must be compared, and finally the protein content of the egg yolk must be considered.

Role of lecithin: As far as the possible effect of the lecithin is concerned, Bloor (5) in noticing the slower removal from the blood of intravenously introduced egg yolk fat suggested that lecithin might be responsible for it. He assumed that lecithin remains in the blood for a long time and by its presence inhibits the removal of the rest of the fat. No conclusive evidence was submitted to support this assumption. Recently Raab (12) injected large amounts of egg yolk intravenously into dogs and found by an analysis of the phospholipids that "it is not possible to produce a prolonged exogenous hyperlecithinemia" in this manner.

We tested the possible role of lecithin in two ways. First we examined the alimentary lipemia of a dog after feeding large amounts of egg yolk. Three grams of egg yolk per kilogram body weight were fed and the total fatty acids in the plasma determined. The initial value was 407 mgm. per cent, it rose to 637 mgm. per cent 2 hours after, and was back to 460 mgm. per cent, nearly the normal level, 5 hours after. These values do not differ from those obtained after olive oil feeding (1), except that the peak and decline in plasma fats are reached in less time, due evidently to the more rapid absorption of the emulsified egg yolk fat.

That lecithin actually appears in large quantities in the thoracic duct chyle after egg yolk feeding was shown by us in dogs with a thoracic duct fistula. Two such dogs were fed by mouth with large amounts of egg yolk (10-12 yolks) 4 hours prior to the operation and chyle collected from the cannulated duct after the dogs recovered from the anesthesia. In the first dog the chyle contained 0.47 per cent lecithin and 1.7 per cent T. F. A. In the second dog the chyle contained 0.32 per cent lecithin and 1.2 per cent T. F. A. In other words the ratio of the fat to lecithin in the chyle after egg yolk feeding is not much different from that which obtains in egg yolk. This in our opinion casts much doubt on the possibility that the presence of lecithin is an important factor in the slow removal from the blood of intravenously injected egg yolk fat.

State of Dispersion: Concerning the state of dispersion of the fat particles we made a comparison of the microscopical picture of our egg yolk emulsions with that of chyle obtained from the thoracic duct after egg yolk feeding. In addition we made an ultramicroscopical examination of the blood plasma at the end of an intravenous egg yolk injection. We have mentioned earlier that in egg yolk emulsions the particles are uniformly distributed and no larger than 2 microns. Particles approaching the size of red blood corpuscles are apt to be strained out by the capillaries of the lungs and liver, as was apparently the case in Raper's experiments. Bloor, repeating some of Raper's work, used casein emulsions of olive oil in which the estimated size was between 2-5 microns. He found that no increase in blood fat could be produced at any time after these injections, although large amounts of fat were introduced.

In chyle we found the particles to be even smaller than in egg yolk. If larger particles are removed faster than smaller ones, then one should expect the egg yolk to be removed from the blood stream more quickly, which is not the case. Ultramicroscopical examination of the blood plasma taken at the height of lipemia following intravenously injected egg yolk shows the same picture as that of normal serum (2 to 3 particles visible in the field). This indicates that the state of dispersion of the egg yolk fat in the blood is similar to that of fat contained normally.

Role of Proteins: The protein content of our egg yolk emulsion was 2-3 per cent calculated on the basis of 16 per cent protein in egg yolk. The protein content of hurger-lymph from the thoracic duct is 3.5 per cent, according to Munk and Rosenstein (10). Thus there is little difference in the amount, although the nature of the proteins and their relation to the fat may be entirely different in the two. We think it is unlikely that the protein present in egg yolk is instrumental in delaying the removal of the fat from the blood, although there is no direct evidence to prove or disprove this contention.

EFFECT OF VARIOUS PHYSIOLOGICAL FACTORS ON LIPEMIA INDUCED INTRAVENOUSLY

In the following experiments the effect of starvation, insulin, glucose, pituitrin, suprarenalin, thyroid substance, parathormone, secretin, cholecystokinin, and bile salts was studied. The lipemia induced intravenously in pancreatectomized, Eck fistula, and thoracic duct fistula dogs was also studied. Experiments were made on the effect of fat feeding by mouth, on the effect of chyle injected intravenously, and the effect of a repeated injection of egg yolk on the lipemia induced by intravenous egg yolk injection. Finally, an experiment was made on the lipemia induced by intravenous chyle administration.

In all these experiments the dogs were not fed 24 hours previously unless otherwise noted. The egg yolk emulsions in saline solution were prepared on the basis of 1.5 gm. egg yolk per kilogram body weight unless otherwise noted. The rate of injection was either 2 cc. or 3 cc. per minute for one hour, the quantity of solution and the rate of injection being determined by the weight of the dog. For the blood analysis blood was drawn at the start, at the end, 2 hours after the end and 5 hours after the end of the experiment unless otherwise stated.

It was observed early in this work that despite widely different experimental conditions the lipemia curves showed little variation from the normal. Therefore more than one experiment was perfomed chiefly in those conditions in which the first observed change was marked.

Starvation: (Table 2.) Dog No. 36, weighing 19 kgm., was starved for 7 days. On the 8th day the routine egg yolk injection was made. The curve obtained in this case was very similar to our normal curve. See Table 1.

Insulin: (Table 3.) Dog No. 19, weighing 13.6 kgm., was given 25 units of insulin subcutneously 45 minutes before the routine egg yolk injection was made. The plasma volumes were 70.5 per cent before, 65.5 per cent at end, 65 per cent 2 hours later, and 68.5 per cent 5 hours later.

Dog No. 30, weighing 19 kgm. was given 30 units insulin subcutaneously 45 minutes before the routine egg yolk injection was made. These curves are not different from the normal, indicating that there is no effect of insulin noticeable.

TABLE 2.

EFFECT OF STARVATION

Dog No.	Total Fatty Acids mgm. in 100 cc. Blood Plasma			
	Before Injection	At End of Injection	2 Hours After End	5 Hours After End
36	196	692	526	471

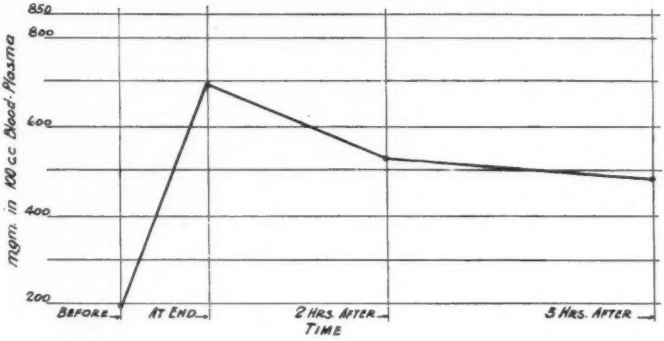
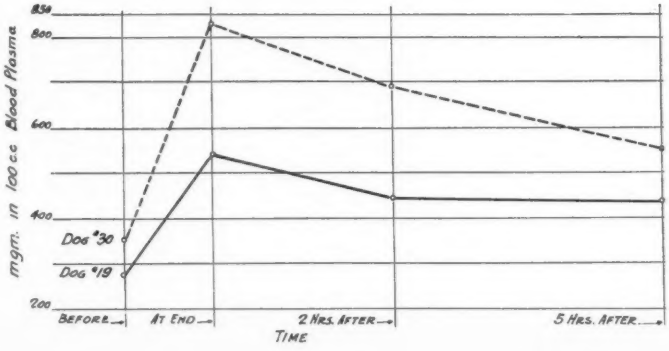


TABLE 3.

EFFECT OF INSULIN

Dog No	Total Fatty Acids mgm. in 100 c.c. Blood Plasma			
	Before Injection	At End of Injection	2 Hours After End	5 Hours After End
19	280	540	443	435
30	360	831	692	554

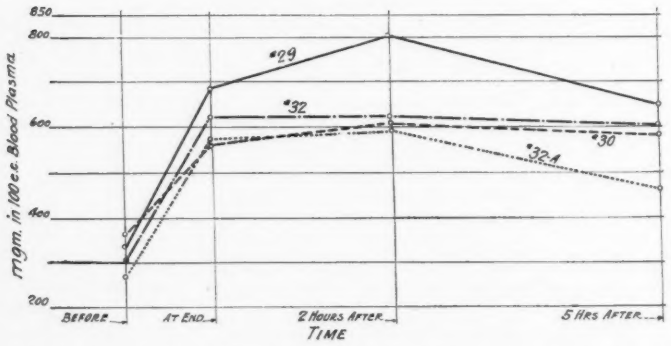


Glucose: (Table 4.) Glucose by mouth and intravenously: Dog No. 29, weighing 19 kgm. was given 50 grams of glucose in 300 cc. water by stomach tube. In this experiment enough glucose was added to the usual egg yolk emulsion to make it 5 per cent. This resulting emulsion was injected in the usual way, one hour later. Glucose by mouth and intravenously: Dog No. 30, weighing 19 kgm. was given 50 grams glucose in 300 cc. of water by stomach tube. In this experiment the egg yolk emulsion was made up to contain 5 per cent glucose and only 0.75 grams egg yolk per kilogram, this being one-half the normal quantity. This emulsion was injected 1 hour later. There was a marked delay in the rate of removal of fat from the blood. Two hours after the end of the injection the fat content of the plasma was higher than at the end, and after

·TABLE·4·

EFFECT OF GLUCOSE

DOG NO	TOTAL FATTY ACIDS mgm in 100 cc. Blood Plasma			
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	5 HOURS AFTER END
29	332	684	806	645
30	368	560	609	570
32	305	728	720	604
32-A	270	582	593.	463



5 hours the fat content was still very high. Glucose by mouth: Dog No. 32, weighing 15.5 kgm. was given 50 grams of glucose in 300 cc. water 50 minutes before the normal routine egg yolk injection was started. Glucose by mouth twice: Dog No. 32 A, same animal as above, was given 50 grams glucose in 200 cc. water by stomach tube 80 minutes before the routine egg yolk injection was started. At the end of the injection 50 grams of glucose in 300 cc. of water was given by stomach tube. Again there was a definite delay in the removal of the fat from the blood.

EFFECT OF (A) SECRETIN, (B) CHOLECYSTOKININ, (C) BILE SALTS. (Table 5.)

(A) *Secretin:* Dog No. 28, weighing 19 kgm. was injected with 50 mgm. secretin, equivalent to 100 threshold doses, added to the usual egg yolk emulsion.

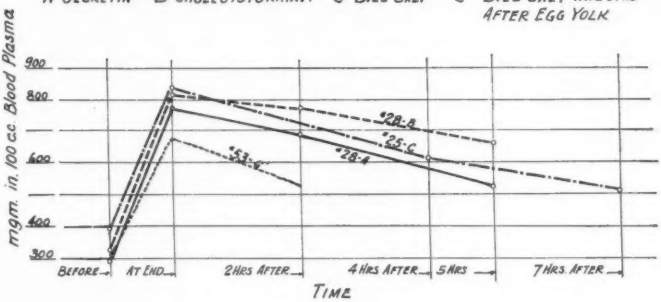
(B) *Cholecystokinin:* Dog No. 28. Same animal as above 5 days later. Cholecystokinin (150 mgm.) equivalent to 50 threshold doses was added to the usual egg yolk emulsion, which was then injected.

·TABLE 5·

EFFECT OF: A)-SECRETIN
B)-CHOLECYSTOKININ
C)-BILE SALTS.

DOG NO.	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma					
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	4 HOURS AFTER END	5 HOURS AFTER END	7 HOURS AFTER END
28-A	299	775	680		525	
28-B	332	814	775		656	
25-C	396	839		609		512
53-C'	305	670	526			

A-SECRETIN - B-CHOLECYSTOKININ. - C-BILE-SALT C'-BILE SALT INJECTED AFTER EGG YOLK

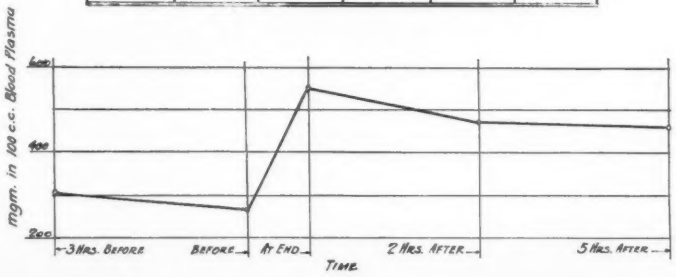


(C) *Bile salts:* Dog No. 25, weighing 11.4 kgm. was injected with 4.5 cc. of a 5 per cent solution of sodium taurocholate (Wilson) added to the standard egg yolk emulsion. Dog No. 53, weighing 10 kgm. after the routine egg yolk injection was finished, was injected at the rate of 2 cc. per minute with 100 cc. of a 2 per cent solution of sodium choleate (Merck). This amount of bile salt

·TABLE 6

EFFECT OF PITUITRIN

DOG NO	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma				
	3 HRS. BEFORE INJECTION	BEFORE INJECTION	AT END OF INJECTION	2 HRS. AFTER INJECTION	5 HRS AFTER INJECTION
21	304	270	550	471	457



represents about one-half the lethal dose. The blood samples showed no hemolysis. The bile salts solution was injected separately to avoid the precipitation of the proteins of the egg yolk, with consequent breaking up of the emulsion. It is seen from a comparison of Tables 1 and 5 that secretin, cholecystokinin, and bile salts did not materially alter the normal lipemia curve in these experiments.

Pituitrin: (Table 6.) Dog No. 21, weighing 10.5 kgm. was given a subcutaneous injection of 10 cc. pituitrin (Lilly, Obstetrical) after a blood sample was taken. Three hours later another 10 cc. dose of pituitrin was injected and the usual egg yolk emulsion injection started. At the end of the injection 10 cc. pituitrin was injected, making a total of 30 cc. The dog remained in good condition throughout the experiment. The plasma volumes were as follows: 63 per cent 3 hours before start; 71 per cent at start; 72.5 per cent at end; 69 per cent 2 hours after end, and 65.5 per cent 5 hours after end. No change from the normal curve was discernible. Pituitrin had no effect on the fasting blood fat value, either, the slight decrease being referable to the dilution as indicated by the plasma volumes.

EFFECT OF (A) SUPRARENALIN, (B) THYROID SUBSTANCE, (C) PARATHORMONE. (Table 7.)

(A) *Suprarenalin:* Dog No. 43, weighing 10 kgm. was given 1 cc. (1:1000) suprarenalin (Armour) subcutaneously at the start of the routine egg yolk injection. At the end of the injection 1 cc. suprarenalin was given and again 2 hours later 1 cc. suprarenalin was given.

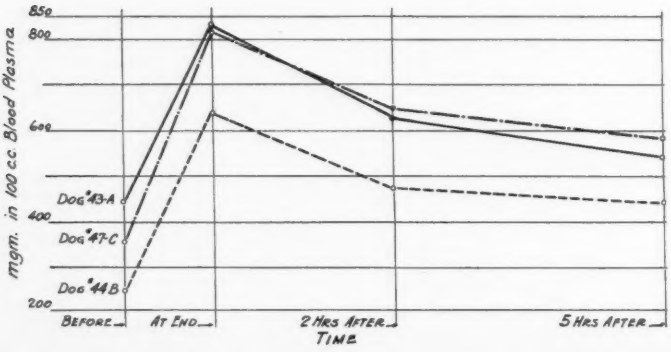
(B) *Thyroid substance:* Dog No. 44, weighing 15.4 kgm. was fed 10 grams of powdered thyroid substance in meat daily for 4 days at the end of which

·TABLE ·7·

EFFECT OF: A) SUPRARENALIN
B) THYROID SUBSTANCE
C) PARA THORMONE

DOG NO.	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma			
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	5 HOURS AFTER END
43-A	446	830	632	540
44-B	249	637	471	440
47-C	360	820	650	581

A-SUPRARENALIN B-THYROID-SUBSTANCE C-PARA THORMONE



time he was highly nervous and irritable. On the 5th day the usual egg yolk injection was carried out.

(C) *Parathormone:* Dog No. 47, weighing 5.7 kgm. was given 20 units parathormone at the beginning and the same amount at the end of the routine egg yolk injection. This dog appeared sick at the end of the experiment and died two days later. It is seen from Table 6 that suprarenalin, thyroid substance and parathormone have no appreciable effect on the lipemia curve in these experiments.

LIPEMIA INDUCED INTRAVENOUSLY IN PANCREATECTOMIZED DOGS. (Table 8.)

Dog No. 31, weighing 13.2 kgm. had a total pancreatectomy. Four days later the urine contained sugar and diacetic acid. On the 5th day the blood sugar was 250 mgm. per cent and the usual egg yolk emulsion was injected. At the end of this experiment the dog was given 20 units insulin subcutaneously, and fed with raw pancreas. Two days after the above experiment, the urine of Dog No. 31* contained sugar and diacetic acid. On the 3rd day, 25 units insulin was given subcutaneously, followed an hour later by the usual egg yolk injection. Five hours after the injection was ended 10 units insulin was given. The blood sugar was 225 mgm. per cent before the insulin, 180 mgm. at the end of the egg yolk injection, 160 mgm. 2 hours after end, 136 mgm. 5 hours after and 47 mgm. 10 hours after end.

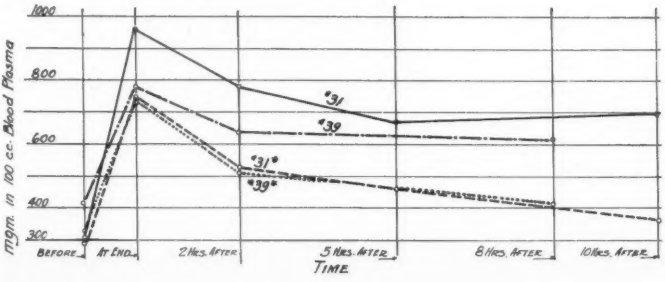
Dog No. 39, weighing 13 kgm. had a total pancreatectomy after which he was given 15 units insulin daily for 3 days. On the 5th day when the urine contained much sugar and the blood sugar was 317 mgm. per cent, the usual egg yolk injection was made. At the end of this experiment and also on the following 4 days 15 units of insulin was given daily and the dog fed with raw pancreas. Dog No. 39,* five days after the above experiment, was given 20 units subcutaneously followed 2 hours later by the usual egg yolk injection. The blood sugar values were 215 mgm. before insulin, 146 mgm. at end of egg yolk injec-

·TABLE·8·

LIPEMIA INDUCED INTRAVENOUSLY IN PANCREATECTOMIZED DOGS.

DOG NO.	TOTAL FATTY ACIDS mgm. in 100 cc. Blood Plasma					
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	5 HOURS AFTER END	8 HOURS AFTER END	10 HOURS AFTER END
31	302	960	775	665		690
31*	296	748	526	465		360
39	415	775	637		609	
39*	316	739	526		410	

* WITH INSULIN



tion, 94 mgm. 3 hours after end and 129 mgm., 8 hours after end. It is seen that in the pancreatectomized dogs the removal of the fat is slower than in our normals. Insulin appears to hasten materially the removal of the fat from the blood in both these dogs.

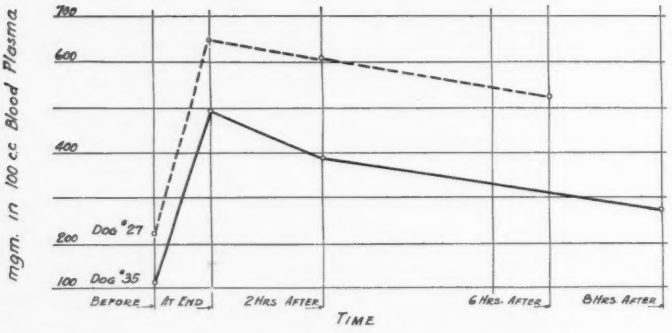
LIPEMIA INDUCED INTRAVENOUSLY IN ECK FISTULA DOGS. (Table 9.)

An Eck fistula operation had been performed 2 months before this experiment on Dog No. 27. The dog was in good condition and weighed 15 kgm. The usual egg yolk injection was made. An Eck fistula operation had been performed

·TABLE·9·

LIPEMIA INDUCED INTRAVENOUSLY IN ECK FISTULA DOGS

DOG NO	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma				
	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	6 HOURS AFTER END	8 HOURS AFTER END
27	222	646	609	526	
35	113	499	382		270



2 months previously on Dog No. 35. The dog was in fair condition and weighed 10.3 kgm. The routine egg yolk injection was carried out. The initial values for the total fatty acids in the blood plasma in these 2 dogs were rather unusually low. The lipemia curves, however, did not differ from our normal curves.

EFFECT OF (A) FAT FEEDING, (B) CHYLE INJECTED INTRAVENOUSLY. (Table 10.)

Fat feeding: (1) Egg yolk by mouth: Dog No. 19, weighing 13.6 kgm. was given 36 grams of egg yolk fed by mouth 20 minutes before the usual egg yolk injection. (2) Olive oil by mouth: Dog No. 23, weighing 9 kgm. was given 15 cc. olive oil fed by stomach tube 15 minutes before the usual egg yolk injection.

Effect of chyle: Dog No. 51, weight 20 kgm. Thoracic duct chyle was obtained from Dog No. 50 after this dog had been fed 12 yolks. Analysis of this chyle showed it to contain 1.2 per cent T. F. A. One gram egg yolk, instead of the usual 1.5 gm. per kilogram was mixed with 180 cc. of this chyle and this emulsion injected intravenously into Dog No. 51. It appears from these experiments that lipemia induced intravenously is not affected by processes accompanying fat digestion and absorption in the bowel. The apparent delay in the removal of the fat from the blood in Dog No. 23 (olive oil) may be accounted for by additional increase in the blood fat due to the alimentary lipemia. Neither did chyle injected with egg yolk hasten the removal of egg yolk fat from the blood.

TABLE 10

EFFECT OF A) FAT FEEDING
B) CHYLE INJECTED INTRAVENOUSLY

DOG NO.	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma				
	BEFORE INJECTION	AT END OF INJECTION	2 HRS. AFTER INJECTION	5 HRS AFTER INJECTION	9 HRS. AFTER INJECTION
19-A	360	714	584	484	
23-B	440	770	665	720	609
51-C	304	775	637		

A- EGG YOLK BY MOUTH — B- OLIVE OIL BY MOUTH — C- CHYLE INTRAVENOUSLY

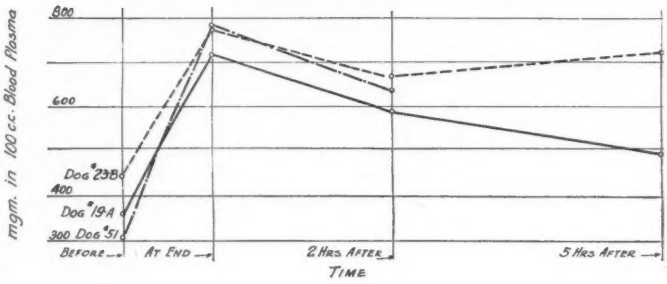
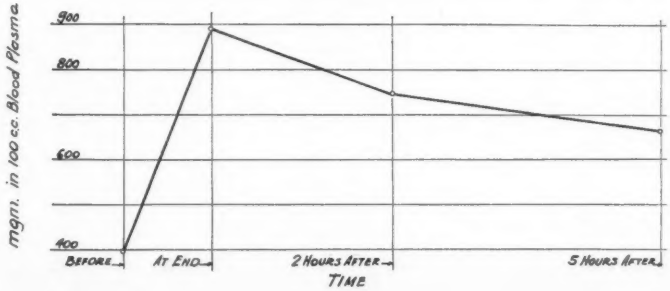


TABLE 11

LIPEMIA INDUCED INTRAVENOUSLY IN A THORACIC DUCT FISTULA DOG FED PREVIOUSLY WITH EGG YOLK.

DOG NO	TOTAL FATTY ACIDS mgm. in 100 cc. Blood Plasma				
	4½ HOURS BEFORE INJECTION	BEFORE INJECTION	AT END OF INJECTION	2 HOURS AFTER END	5 HOURS AFTER END
40	399	399	885	740	665



LIPEMIA INDUCED INTRAVENOUSLY IN THORACIC DUCT FISTULA DOG FED PREVIOUSLY WITH EGG YOLK. (Table 11.)

Dog No. 40, weighing 15.25 kgm. was fed 60 cc. olive oil by stomach tube and the thoracic duct cannulated 4 hours later under ether anesthesia. The next day the dog was fed 5 egg yolks, about 90 grams, in 300 cc. saline solution by stomach tube. Four and one-half hours later the flow of cream-like chyle was observed and the usual egg yolk injection was started. It is seen from Table 11 that no increase in the blood fat could be observed 4½ hours after fat was fed to this thoracic duct fistula dog and that digestion and absorption of fat in the bowel did not affect the lipemia induced intravenously in this dog. This was repeated in Dog No. 56.

TABLE 12

LIPEMIA INDUCED BY INTRAVENOUS CHYLE ADMINISTRATION

DOG NO	TOTAL FATTY ACIDS mgm in 100 cc Blood Plasma			
	BEFORE INJECTION	AT END OF INJECTION	2⅔ HRS. AFTER INJECTION	5⅓ HRS AFTER INJECTION
41	368	388	360	330

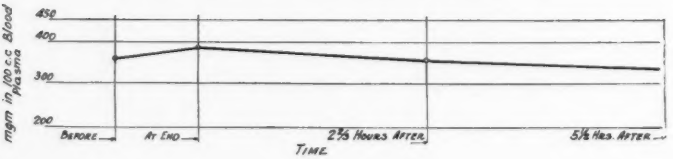
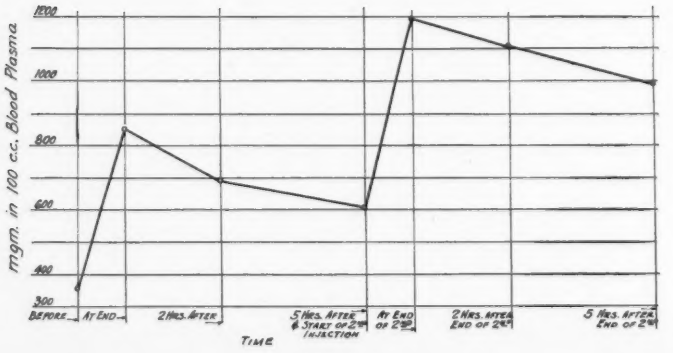


TABLE 13

EFFECT OF A REPEATED INJECTION OF EGG YOLK EMULSION

DOG No.	TOTAL FATTY ACIDS mgm. in 100 c.c. Blood Plasma						
	BEFORE FIRST INJECTION	AT END OF FIRST INJECTION	2 HRS AFTER END OF FIRST INJECTION	5 HRS AFTER END OF 1ST & START OF 2ND INJECTION	END OF SECOND INJECTION	2 HRS AFTER END OF 2ND INJECTION	5 HRS AFTER END OF 2ND INJECTION
30	360	850	692	609	1190	1110	970



LIPEMIA INDUCED BY INTRAVENOUS CHYLE ADMINISTRATION. (Table 12.)

Dog No. 41, weighing 9.1 kgm. was injected at the rate of 2 cc. per minute with 100 cc. chyle (obtained from a thoracic duct fistula dog fed previously with egg yolk) containing 1.7 per cent T. F. A. equivalent to 0.2 gram fat per kilogram. Hardly any increase of the blood fat was noticeable at the end of the injection. Soon the initial value was restored and later an actual decrease was observed. This was repeated in Dog No. 55.

EFFECT OF A REPEATED INJECTION OF EGG YOLK EMULSION. (Table 13.)

Five hours after the end of a routine egg yolk injection Dog No. 30, weighing 19 kgm. was given a second injection of the same amount of egg yolk. Table 13 shows that the lipemia caused by the second injection of egg yolk is not influenced by the previous injection and gives a typical curve simply superimposed upon the first curve.

DISCUSSION

It has been pointed out in a previous paragraph that the removal of egg yolk fat from the blood is much faster if the fat passes the bowel than if the fat is injected intravenously. Further experiments indicated that outside of this difference of quantitative nature there is a more fundamental qualitative difference between these two processes. Thus we found that neither starvation nor insulin has any appreciable effect on intravenously induced egg yolk lipemia (Tables 2 and 3), while starvation promotes and insulin inhibits alimentary olive oil lipemia, as has been shown in a previous paper (1). Such different response to the same factor is still more conspicuous when we compare the effect of glucose. We found that glucose definitely delays the removal of intravenously introduced egg yolk fat from the blood (Table 4), while it markedly hastens the transport through the blood of fat which was absorbed from the alimentary canal (1).

We mentioned that there is no evidence to show that either the size of the particles in the yolk emulsion or its lecithin content or its protein content could be responsible for the difference in the rate of removal. It is still more difficult to account for the qualitative differences just mentioned by assuming causative importance of these factors. Rather, one is led to the conclusion that the difference may be due to some change which occurs either to the fat or otherwise in connection with the passage of the fat from the bowel, a change which facilitates the removal of the fat from the blood in alimentary lipemia but does not take place when fat is introduced intravenously. Or, in other words, the intestinal mucosa acts on fats in such a manner either physically or chemically, or in both ways as to cause it to be deposited rapidly in the tissues.

We carried out a number of experiments in trying to determine the nature of this supposed change, the idea being that by the addition of this change it would be possible to produce intravenous egg yolk lipemia with the quantitative and qualitative features of alimentary lipemia. Practically this means that along with the intravenous egg yolk injection we applied certain factors known or supposed to be operative during the digestion and absorption of fat in the alimentary canal, and investigated

whether it is possible to increase materially the rate of removal from the blood of the fat by this means.

It is known that the appearance of fat in the alimentary canal is followed by increased production of secretin, cholecystokinin and bile. We found, however, that the addition of these substances does not hasten the removal of injected egg yolk fat (Table 5).

We then investigated the possible role of pituitrin which is capable of causing, according to Coope and Chamberlain (13), an accumulation of fat in the liver in rabbits, and according to Raab (14) a decrease of the blood fat of dogs. In our experiment, pituitrin—in amount similar to that applied by the authors just quoted—had no effect on the fasting total fatty acid value of the blood, nor on the lipemia curve after intravenous yolk fat administration (Table 6). Other hormones, suprarenalin, thyroid substance and parathormone were examined, also with negative results (Table 7).

The role of the pancreas was then investigated by using pancreatectomized dogs. We found that intravenously introduced yolk fat is removed at a slower rate from the blood of such animals as compared with normal dogs, although the difference is not very striking. We also found that insulin, which has no effect in normal dogs (Table 3), reduces rather markedly the lipemia in pancreatectomized dogs (Table 8). We are quite unable to explain this phenomena at the present time.

It is known that after fat feeding variable but usually large amounts of the ingested fat are taken up by the liver. We sought information on the role of the liver in the removal of intravenously introduced fat from the blood by experimenting upon Eck fistula dogs. We found that elimination of the liver to the extent known to exist in Eck fistula dogs does not materially affect the lipemia in question (Table 9).

The possibility of the importance of some hitherto unrecognized substances or hormones operative in alimentary fat absorption was investigated by us in experiments in which the intravenous egg yolk injection was accompanied by previous fat feeding. Comparatively small amounts of fat were fed in these experiments to avoid marked alimentary lipemia. It was found that under these conditions the intravenously introduced yolk fat did not leave the blood stream any faster than in our standard experiments (Table 10). In order to test the effect of fat feeding when a large amount of fat is fed, intravenous experiments were carried out on two thoracic duct fistula dogs fed previously with a large fat meal. No fat reached the blood stream through the thoracic duct in the test, while hormonal or other substances produced during fat absorption were not prevented from passing into the blood circulation. However, the rate of fat removal from the blood in these experiments was not different from the standard (Table 11).

It still remained possible that substances instrumental in fat removal from the blood following alimentary fat absorption could be carried by the

lymph circulation instead of the blood stream. To study this possibility we injected thoracic duct chyle together with egg yolk intravenously. Again no difference from the standard was noted (Table 10). However, when chyle obtained from a thoracic duct fistula dog fed previously with egg yolk was injected into the vein of a normal dog the fat disappeared rapidly from the blood (Table 12), as was predicted from our alimentary lipemia experiment with egg yolk feeding.

Finally a repeated injection of egg yolk fat was made in analogy to the method of repeated glucose administration used by Traugott (15) and others to demonstrate the response of the sugar regulating mechanism. We found that the removal of intravenously injected egg yolk fat was not stimulated by a previous injection (Table 13).

We recall that with equal amounts of *sugar* administered, hyperglycemia is much more pronounced following intravenous injection than oral administration (17). The faster removal of sugar following intestinal absorption was explained by assuming a more prompt response of insulin production. However, the question why fat absorbed from the alimentary canal is removed from the blood so rapidly in contrast with the slow removal of intravenously injected yolk fat remains open. We feel that a hormone mechanism is not concerned, but that the intestinal mucosa acts on the fat itself and renders it more readily taken up by the tissues.

There is some evidence based on different types of experiments to support this contention. Leathes and Raper (16) mention that Munk and Rosenstein observed that the chyle fat from their patient with the lymph fistula had a lower melting point than the food fat when mutton fat was present in the food. They also quote Arnschink, Franck, and later Bloor, as having made similar observations on dogs of changes in the melting point and iodine value with different fats being fed. Leathes and Raper conclude that, "It is possible that fat may undergo some modification during absorption, so that the fatty acids resulting from the hydrolysis of the fat in the food are recombined into glycerides, but not exactly in the same way, so that a fat different from that in the food results." We have some experimental evidence, to be submitted later, which indicates that certain physical changes which the fat undergoes in the bowel are of paramount importance.

SUMMARY

1. Emulsions of egg yolk were injected intravenously into dogs at a slow constant rate, and the resulting lipemia studied.

2. It was noted that there is a sharp rise in the total fatty acid content of the plasma at the end of the injection, followed by a slow disappearance in the succeeding hours.

3. The rate of removal from the blood of fat thus introduced is much slower than that of fat absorbed from the intestines.

4. Starvation, insulin, pituitrin, suprarenalin, thyroid substance, parathormone, secretin, cholecystokinin, and bile salts have no effect on the rate of removal.

5. Glucose has a definite effect in delaying the removal.

6. In pancreatectomized dogs the removal of intravenously injected yolk fat is slower and insulin hastens the removal in these animals.

7. In Eck fistula dogs the lipemia curve is not different from the standard.

8. Previous fat feeding in normal dogs or in a thoracic duct fistula dog or simultaneous chyle injection in a normal dog had no effect.

9. Chyle fat of a thoracic duct fistula dog previously fed with egg yolk disappears rapidly from the blood stream when the chyle is injected into the vein of a normal dog.

10. The lipemia caused by a second injection of egg yolk was not influenced by the previous injection.

11. We feel that a hormone mechanism is not concerned, but that the intestinal mucosa acts on the fat itself and renders it more readily taken up by the tissues.

We desire to thank Dr. A. C. Ivy for his kindly aid and advice in this work, and to thank Dr. L. A. Crandall, Jr., for the use of his Eck fistula dogs.

BIBLIOGRAPHY

1. Rony and Ching: This Journal, **14**: 355. 1930.
2. Bondi and Neumann: Wien. Klin. Wchnschr. **23**: 734. 1910.
3. Schott: Deutscher Arch. f. klin. Med. **112**: 403. 1913.
4. Raper: J. Biol. Chem. **14**: 117. 1913.
5. Bloor: J. Biol. Chem. **19**: 1. 1914.
6. Saxl and Donath: Wien. Arch. f. inn. Med. **13**: 7. 1927.
7. Markowitz and Mann: Am. J. Physiol. **93**: 521. 1930.
8. Bloor: J. Biol. Chem. **77**: 53. 1928.
9. Bloor: J. Biol. Chem. **82**: 273. 1929.
10. Munk and Rosenstein: Virchows Arch. f. path. Anat. **123**: 230. 1890.
11. Rony and Levy: J. Lab. & Clin. Med. **15**: 221. 1929.
12. Raab: This Journal, **14**: 150. 1930.
13. Coope and Chamberlain: J. Physiol. **60**: 69. 1925.
14. Raab: Ztschr. f. d. ges. exper. Med. **49**: 179. 1926.
15. Traugott: Klin. Wchnschr. **1**: 892. 1922.
16. Leathes and Raper: The Fats, 1925. Longmans, Green and Co., London. P. 132.
17. Lennox: J. Biol. Chem. **73**: 237. 1927.

THE PITUITARY-GONADAL RELATIONSHIP AND THE PROBLEM OF PRECOCIOUS SEXUAL MATURITY*

EARL T. ENGLE

Department of Anatomy, College of Physicians and Surgeons, Columbia University

NEW YORK

An attempt is made in the following pages to present a concept of the pituitary-gonadal complex involved in the development of puberty and sexual maturity in experimental animals.

The problem is discussed only in relation to the female, although data are available to warrant the conclusion that the principles are not different in the male. The superficial characters of the female, such as the rupture of the vaginal plate in rodents, with subsequent observation of the vaginal smear, make the successive stages of development more easily determined than in the male rodent.

The rupture of the vaginal plate has been for some years accepted as a sign of sexual maturity. Usually, the first oestrus was considered in relation to the establishment of the vaginal orifice, and together were regarded as sexual maturity. Long and Evans (1) take as their standard the functional capacity of the animal to rear a first litter. In the recent paper by Mirskaia and Crew (2), puberty is defined as "that stage of individual development characterized by the ability to elaborate functional gametes and by the physical ability to and the desire to play the appropriate role in mating." Maturity is dependent upon the ability to produce offspring and bear them, and by "the exhibition of maximum fertility ratio." While it is thought that the requirement expressed by the last quoted phrase is not essential, and quite impossible for first litters, the statements do definitely separate the two physiological conditions into their proper categories. Except that puberty appears to be a slowly developing process, and cannot be considered as an event to be specifically dated, as will be discussed below, the definitions as stated by Mirskaia and Crew are accepted here.

In general, however, the earlier data are concerned primarily with relation of first oestrus to the establishment of the vaginal orifice, and incidentally to first mating.

Long and Evans report that in 200 rats the range of vaginal opening was from 34 to 109 days with an average of 72 days. Evans and Bishop (3) state that in another series of 570 rats, the first oestrus occurs within a day or two after the rupture of the vaginal plate, ranging from 32 to

*Read before the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 9, 1931.

Aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

94 days, with 86.6 per cent occurring between 37 and 54 days. Stone (4), analyzing the data of Evans, for 325 of these animals found an average of 1.49 days between rupture of plate and first oestrus. In Stone's series 80 per cent of the first oestrus cycles occurred between the 37th and the 52nd day. Long and Evans record that of 193 rats examined, the first ovulation occurred simultaneously with vaginal opening in 46 per cent and within 5 days in 68 per cent. Further they state that there is "about 10 or 12 days between the average age at first oestrus and the average age at first coitus." In their series the vaginal plate was at 76.5, the first coitus at 92.7 days.

Similar observations have been made on mice, though with fewer numbers involved. Engle and Rosasco (5), reporting on the age distributions of 100 female mice at the rupture of the vaginal plate, found a range of from 28 to 49 days, with a median at 35. Mirskaia and Crew (2), reporting on vaginal opening, first oestrus, first mating and pregnancy after first matings, include data from 100 female mice of two genetic types. Their data are discussed below. MacDowell and Lord (6) record the age at first oestrus of a few mice with a range of 51 to 85 days, which averaged 65.3.

When all of these data are considered, it is apparent that, in the normally developing mouse or rat, the first oestrus occurs within 48 hours in the majority of the animals, and ovulation is synchronous with first oestrus probably in less than half of them. There is then a lag of some days before the first copulation, and it is known that the proportion of term pregnancies following first matings is lower than for adult animals.

These manifestations of puberty, the rupture of the vaginal plate, the first oestrus, first ovulation and first mating have been studied in normally developing animals and are considered as the several phases of the period of puberty. When by the use of some body fluid, tissue or tissue extract, certain of these pubertal phenomena were caused to appear before the normal age, the current clinical term, "precocious sexual maturity," was applied, and new methods and new standards of experiment became accepted.

The interpretation of the earlier experiments dealing with problems of gonadal physiology has been adequately reviewed by Lipschütz (7, 8, 9).

Allen and Doisy (10) in showing that the factor causing rupture of the vaginal plate and vaginal cornification was contained in the liquor folliculi, spoke of the condition as "precocious sexual maturity." Later Philip E. Smith (11) demonstrated the next step in this physiological process when he showed the presence in the anterior lobe of the pituitary of the hitherto unknown factor involved in ovarian growth and development. In the subsequent papers [Smith (12), Smith and Engle (13)] these phenomena were again referred to as "precocious sexual maturity," explaining that, "by sexual maturity we mean the appearance of the first oestrus. In mice in which sexual maturity is induced, mating, in all cases where

tested, followed within 24 hours of the vaginal opening. . . . Hence, the term puberty and sexual maturity are, for our purpose, synonymous."

Zondek and Aschheim (14) in 1927 and many later papers reported experiments of the same nature and likewise designated the response as "sexuelle Frühreife."

The first challenges of the usage of the term "sexual maturity" were expressed by Siegmund (15) and Ehrhardt (16) in 1928. The latter, working in Seitz' laboratory, reported that the mice which had received the anterior lobe transplants did not deliver a first litter earlier than normally maturing controls. Seitz (17) considers that the phenomena under discussion might be called a morphological rather than a functional maturity.

In our early experiments on the action of the anterior pituitary on the gonads and on somatic structure, we were obliged to sacrifice our precociously developing animals for histological examination (Smith and Engle). Shortly after, however, attempts to obtain uterine implantations of the ova from cases of induced superovulation in these immature animals were made. This was possible in a few cases in adult animals [Engle (18)]. The greatest number of embryos in the uterus of an adult animal was 29. Although the experiments were not extensive in number, it should be mentioned that no female delivered an excessive number of young in a litter. It was known that the ova were implanted in those cases which were autopsied prior to the 14th day, and it was assumed that conditions of overcrowding led to the death and resorption or abortion of the conceptuses in the other instances. From time to time since those earlier attempts, implants have been made into immature mice, and their course was followed after mating. The results were not conclusive, however, and the present experiment represents a series of attempts to study the mating and reproductive capacities of these precociously developed animals.

While the data here reported were being collected, the experiments of Mirskaia and Crew (19) were published. Immature mice were given unmodified urine of pregnancy twice daily for 3 days, in the manner which is routine for the Aschheim-Zondek test. Oestrus appeared in the injected animals on the 4th to 6th day of the experiments, and a few of the animals mated. In no case did pregnancy ensue. Mirskaia and Crew do not regard the occurrence of ovulation as essential to the interpretation of their results. Professor Zondek is quoted in a special communication to the authors: "Die injection von schwangeren Urin ruft bei infantilen Maus Ovulation hervor." As stated in a previous paper [Engle (20)] we have been unable to induce ovulation in the mouse after treatment with the urine of pregnancy, and still do not regard the complex of substances in whole urine as carrying the same factors which are transmitted by means of implanted anterior lobe. These authors assume that since pregnancy did not follow any mating in precociously developed mice,

they were dealing with a condition of genital maturity in an immature soma. Even though their conclusions appeared quite valid, it seemed advisable to continue the investigations using a method which was known to cause ovulation in a certain, though unknown, proportion of mice, and to examine microscopically a few of the animals to determine the incidence of ovulation and fertilization.

EXPERIMENTAL METHODS AND DATA

The group of mice included in this experimental group number 72. Of this number 63 were given implants of the anterior lobe daily on 3 successive days, with the exception of 11 animals in which the glands were implanted on the 1st, 2nd and 4th day of treatment. The animals were between 17 and 20 days of age and weighed from 6 to 10 grams. Without exception the rupture of the vaginal plate occurred at the end of the 3rd day or on the 4th day. After the 3rd day of treatment these mice were kept with vigorous, mature males.

Thirty of the animals which mated at this first induced cycle were killed sometime during the 5 days after mating and the ovaries examined at autopsy. Those with corpora lutea and a few doubtful ones were serially sectioned, together with their tubes or tubes and uteri, depending on the number of elapsed days since copulation.

An examination of Table 1, in which these data are presented in detail, shows that in this group mating occurred on the day of rupture of the vaginal plate in 11, or one-third of the cases; on the 1st day after

TABLE I

MICE WHICH MATED AT INDUCED OESTRUS AND SACRIFICED TO DETERMINE OVULATION

No. Mouse	Weight Gms.	Age	A. P. Implants		Mating After Vaginal Orifice		Age At Plug	Ovulation + —
			No. Daily	No. Days	+ —	Days		
2590	6+	17	1	3	+	3	24	—
2606	6	18	1	3	+	0	21	+
2607	6	18	1	3	+	0	21	—
2608	6	18	1	2	+	1	22	+
2610	5	18	1	3	+	2	23	—
2612	8	19	1	3	+	6	26	+
2615	8	18	1	4	+	0	22	+
2618	7	18	1	3	+	4	23	—
2619	7	18	1	3	+	3	24	—
2620	8	18	1	3	+	2	23	—
2663	8	19	1	4	+	4	27	—
2664	8	19	1	3	+	0	22	—
2665	9	18	1	3	+	0	21	—
2666	9	18	1	3	+	2	23	—
2669	8	17	1	4	+	1	22	—
2674	8	17	1	2	+	0*	20	—
2676	8	17	2	2	+	0	20	—
2678	8	17	1	4	+	1	21	—
2680	8	17	1	4	+	1	21	—
2689	10	20	2	3	+	0*	25	+
2694	9+	18	1	3	+	0	23	+
2695	9+	18	1	3	+	5*	28	+
2699	8	18	1	2	+	1	22	—
2701	8	18	1	2	+	1	22	+
2716	7	18	1	3	+	1	22	+
2720	8	18	1	3	+	0	22	+
2730	7.19	17	1	3	+	1*	20	—
2731	7.49	17	1	3	+	1	20	—
2732	9.49	17	1	3	+	0*	20	—
2733	9.3	17	1	3	+	2	22	—

*These animals mated on the day recorded, and on more than one successive days.

the rupture in 9, and between the 2nd and 6th days in the remaining 10. Thus two-thirds mated within 24 hours after the establishment of the vaginal orifice. Copulation plugs were found for 5 of these animals on the 1st day and one or more succeeding days of mating. M2695 did not mate until 5 days after the rupture of the plate, and then mated on 4 successive days. M2674 mated on the 1st day and on 3 successive days. In these cases, as with all, the male had been with the female from the day before the opening of the orifice.

Of these 30 animals which were autopsied, none was sacrificed earlier than 1 day nor later than 5 days after the mating, or in the cases of multiple matings, after the 1st mating. Eleven, or slightly more than one-third, were found to have ovulated. In some cases where 2 or 3 days had elapsed before autopsy no eggs were to be found, the tube being full of detritus as in unmated animals killed after ovulation. In these cases the corpora were carefully examined, and corpora with retained ova were not found in numbers greater than occurs in normal animals. In other cases 15 to 20 morulae or blastocysts were counted in one tube or uterine horn and checked against the number of corpora lutea. In all cases there was considerable discrepancy, showing that many eggs either had not been fertilized or had degenerated following fertilization. Many of these developing eggs had been fertilized but at the time of examination were beginning to fragment. On the other hand there were found in animals killed at the appropriate stages, many morulae in the tubes, and blastocyst stages within the uterine lumen, which were, so far as could be decided, quite normal, yet none were implanted.*

Of the 5 animals which had mated more than once, 3 did not ovulate. Seven animals mated on the 1st to 5th day after establishment of the vaginal orifice, but were not sacrificed (Table II). If any eggs were fertilized following mating, no animal gave a positive pregnancy sign on the 12th, 13th or 14th days, as is constant in pregnant mice. The later histories of these mice is discussed below.

As will be noticed, only a few of these treated animals had a single day with cornified cells in the smear, the majority having 2 days, and a few 5 days with uninterrupted cornified smear. Yet, taking the extended cornification as an indication of continued ovarian action, 26 animals did not mate at this first induced cycle, though the males were known to be of satisfactory aggressiveness and potency. It is true that one or two may have mated without the plug having been found.

*The recent work of Smith and White (J. A. M. A., 1931, in press) has demonstrated that the action of a factor of the anterior lobe is necessary for the proper formation and the proper functioning of the corpus luteum. This new datum taken in conjunction with the work of Corner and Allen affords a very interesting means by which these phenomena are to be explained. Since, as reported above, the fertilizd eggs of immature mice were found in morula and blastocyst stages before extensive degeneration had occurred, I have sought for an explanation involving the corpus luteum. The post-ovulation corpus is histologically similar in these animals to that of mature mice. In the light of the work of Smith and White, showing that the anterior lobe is essential to the maintenance and functional activity of the corpus, we offer as a tentative solution the hypothesis that the anterior lobe of these immature animals, which is known to be functionally inadequate, fails to assist the corpus in the elaboration of the progestational factor. As has been shown by Shelesnyak, the uterus itself is capable of responding under proper sensitization.

TABLE II

MICE WHICH MATED AT THE FIRST INDUCED OESTRUS AND CONTINUED TO FIRST NATURAL CYCLE

No. Animal	Weight Gms.	Age Days	A. P. Implants		Vaginal Orifice + —	First Oestrus Age, Days	No. Days Corns	First Plug Induced Oestrus	First Normal Oestrus Age, Days	Age First Mating After Normal Oestrus	Pregnancy	
			No. Daily	No. Days							R. B. C.	Litter
2717	8	18	1	3	+	23	4	27	37	37	+	+
2719	8	18	1	3	+	21	3	24	41	49	+	+
2721	9	18	1	3	+	21	1	21	27	27	+	Dead
2729	7	17	1	3	+	20	5	26	No R. B. C.	no oestrus	at 58th day	
2734	6	19	1	3	+	22	1	27	No R. B. C.	no oestrus	at 59th day	
2735	5.6	19	1	3	+	22	5	27	No R. B. C.	no oestrus	at 59th day	
2739	8.6	19	1	3	+	21	2	22	No R. B. C.	no oestrus	at 58th day	

TABLE III

Mice which Did Not Mate at Induced Oestrus

No. Animal	Weight Gms.	Age Days	A. P. Implants		Vaginal Orifice + —	First Oestrus Age, Days	No. Days Cornified	Second Oestrus Age, Days	First Plug Age, Days	Pregnancy	
			No. Daily	No. Days						R. B. C	Litter
2592	..	17	1	3	+	21	2	60	60	+	+
2593	..	17	1	3	+	21	2	36	36	+	+
2609	5	18	1	3	+	21	1	41	42	+	+
2611	8	19	1	3	+	22	5	48	48	+	+
2613	8	19	1	3	+	22	4	52	52	—	—
2614	8	18	1	4	+	22	1	44	44	+	—
2616	8	18	1	4	+	22	5	52	52	—	—
2668	8	17	1	4	+	21	2	41	43	—	—
2670	6	17	1	3	+	20	6	42	42	+	..
2671	6	17	1	3	+	20	2	44	49	+	..
2672	6	17	1	3	+	20	3	42	42	+	..
2675	8	17	2	2	+	20	2	42	42	+	..
2677	8	17	2	2	+	20	1	42	42	+	..
2679	8	17	1	4	+	20	3	49	49	+	..
2682	..	17	1	4	+	20	1	46	No plug at 119	days	
2683	..	17	1	4	+	20	1	46	47	+	+
2688	10	20	2	3	+	23	2	40	51	—	—
2693	9	19	1	3	+	23	1	39	No plug at 59	days	
2718	8	18	1	3	+	21	2	40	44	+	+
2722	8	18	1	3	+	21	2	41	No plug at 53	days	
2755	9.9	19	1	3	+	23	2	No cycle 41	days		
2756	10.5	19	1	3	+	23	2	No cycle 41	days		
2757	9.5	19	1	3	+	23	2	No cycle 41	days		
2758	9.4	25	1	2	+	27	2	No cycle 46	days		
2759	9.4	25	1	2	+	27	2	44	No plug at 46	days	
2760	8.4	25	1	2	+	27	2	No cycle 46	days		

Since mating had failed after this initial oestrus, the animals were kept in cages with the male, 2 or 3 females being with 1 male, and daily smears taken. The second cycle or the first natural cycle occurred at ages ranging from the 37th to the 60th day, with the average at 43.8 days (Table III, Fig. 1). Mating occurred during this oestrus period in less than half the cases.

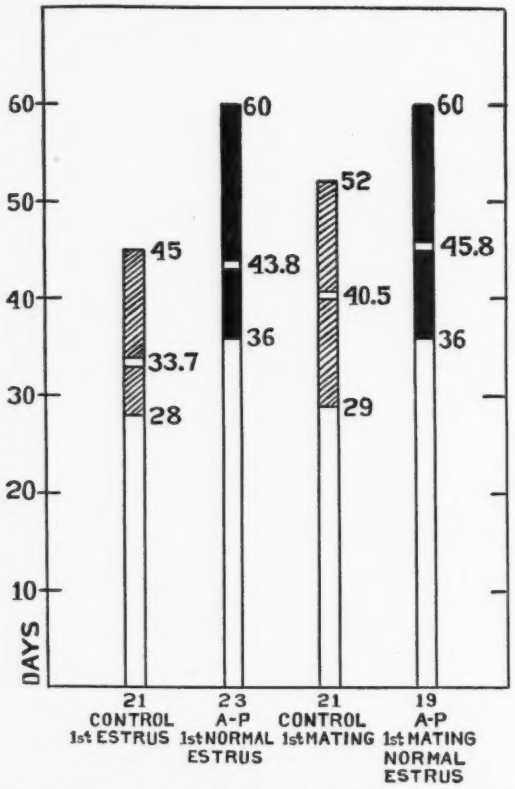


Figure 1. The average and range of ages for first oestrus and first mating for anterior pituitary treated and control mice.

The average age of the animals at first mating was 45.8 days, although the average age of the first matings which were later proven fertile was 44.9 days.

In general then, in this group in which mating did not occur at the first induced cycle, the response was delayed beyond that for the control animals (See Fig. 1). The range of ages is greater, and the average age at first mating is higher than for the control animals. While the number of animals is small, it is probable that this delay does have some significance. It gives some basis for thinking that the normal pituitary-gonadal

relationship has been disturbed by the pituitary implants, and that the normal maturation of the organism has been modified.

In the light of recent reports regarding an inhibiting effect of oestrin on the anterior lobe, a small series of animals (Table IV) were given a

TABLE IV
MICE INJECTED WITH AMNIOTIN

No. Animal	Weight Gms.	Age Days	Treatment	Days	Age V. O.	First Normal Oestrus	First Plug	Pregnancy + —
2724	9	20	Amniotin .1 cc.	3x3	23	33	34	—
2725	9	20	Amniotin .2 cc.	3x3	23	36	37	+
2743	7	17	Amniotin .1 cc.	2x3	20	38	41	+
2744	8.7	17	Amniotin .1 cc.	2x3	20	48	48	—
2748	15.9	31	Amniotin .1 cc.	2x3	32	41	41	+
2749	16	31	Amniotin .1 cc.	3x3	32	?	49	—
2746	12.8	32	Amniotin .1 cc.	3x3	33	40	40	+
2752	10.4	25	Amniotin .1 cc.	2x3	27	38	38	+
2754	10.6	25	Amniotin .1 cc.	2x3	27	None	at 46 days.	

course of Amniotin over a short period of time and observed for the succeeding first oestrus and mating. The Amniotin* was given in doses of 0.1 cc. 3 times daily for 3 days. This, according to the assay of the manufacturers, is a unitage of 2 rat units 3 times daily, or a total of 18 rat units. In certain cases this amount was given twice daily for 3 days, a total of 12 units. Even so large a dosage was not adequate to produce a mating response in any of this small series. The first normal oestrus appeared between the 33rd and 48th days, with an average age of 41 days at the first plug. Seven of the 9 initial matings were fertile.

Since there was no noticeable variation in response of these Amniotin animals, either from the other experimental mice or from the normal ones, a second group was not run. In so far as influencing reproductive capacity, this type and amount of treatment was ineffective in this small series.

DISCUSSION

The use of the term, "precocious sexual maturity," as protected by the definitions given at the time by Allen, Smith, and Smith and Engle, was not unjustified. In the light of more data, however, it has been necessary to modify the definition to express the broader concept. The mice reported in this series of experiments cannot be considered to be sexually mature and in the strict sense the term, "precocious sexual maturity," should not be used, because in these animals no regularly recurring cycles are maintained, experimentally produced ovulations are not a constant feature, nor do all of the animals mate at the induced cycles. The only report of maintenance of cycles in precociously developing animals is that of Frank, Kingery and Gustavson (21), who caused oestrual changes in young rats by injections of female sex hormone. They concluded that within 5 to 7 days after the induction of these precocious uterine and

*The Amniotin was furnished through the kindness of Dr. J. F. Anderson, Director, Research and Biological Laboratories, E. R. Squibb & Sons.

vaginal changes, "the artificially produced puberty may be followed by spontaneous, regular cyclic phenomena," and also state that, "if the threshold of puberty is once crossed, sexual maturity may be maintained spontaneously in immature rats." Frank (22) again states that some of these immature injected animals may "remain adult and continue ovulating cyclically." Some doubt is expressed by Frank in his recent volume as to the causation of the cyclic phenomena, when he suggests that the continuance of function may have been due to "the presence of unsuspected anterior lobe pituitary extract." Even though his animals were older than the rats commonly used in this type of experiment, this report constitutes a unique instance in the work on the hormones involved in the puberty reaction.

The failure to produce a true sexual maturity in the young animal has broadened the scope and increased the complexity of the problem. Though it is recognized that a factor from the anterior lobe is the main stimulus in causing ovulation, the manner in which this is effected is not clear. If too little is administered, the folicle apparently does not reach the proper degree of development; if too much, the follicle becomes cystic, and the ovum atretic. The method of administration has been demonstrated by Friedman (23) to be important in the rabbit.

Unlike the experiments reported by Mirskaia and Crew, ovulation in the animals here reported is known to occur and fertilized ova were found, although no uterine implantations were observed. When no uterine implantations of ova were found, it was considered advisable to test the physiological condition of the uterus in treated animals. One phase of this work had already been done by Smith (12), who found that the uterine strip of the precociously developed rat gave the same type of kymographic tracing as that of the normal adult rat in oestrus. Accordingly, Shelesnyak (24) studied the capacity of the uterus of treated immature rats to produce deciduomata after stimulation with thread loops, after the technic devised by Long and Evans. Immature rats, 18 and 19 days of age, were given anterior lobe implants for 3 days, following which they received injections of the basic extract of beef pituitary for an additional 3 or 4 days. At this time the uteri were stimulated and injections maintained until autopsy 4 days later. In all of these animals macroscopic deciduomata were observed. In the control series, which had been given injections of estrin (Amniotin-Squibb) in place of the anterior lobe implants and followed by the injections of basic extracts, no deciduomatus reaction occurred.

The deciduomata response may not in every respect be fully comparable to the changes caused by the developing corpus luteum and blastocyst. The uniformly positive response gained here should indicate, however, that the failure of fertilized eggs to attain uterine fixation is not due solely to an inadequate maturation of the endometrium, as was postulated by Mirskaia and Crew.

From this series and from the reports of others cited above, it is known that mating may take place in these mice without ovulation following. It is not known whether ovulation may occur in those instances where oestrus was not accompanied by mating. Weisner and Mirskaia (25) have discussed the amount of oestrin prepared by them from pregnancy urine, which is required to produce the mating response in castrated mice. In their experiments, cornification occurred in all injected animals, but 9 animals did not mate, even after injection of as much as 280 mouse units. As in our own series, mating occurred on successive days in several animals.

The occurrence of cornification of more than one day's duration (ranging from 1 to 5 days) following anterior pituitary implants is also similar to the observation of Weisner and Mirskaia. In these immature animals both the mating response and the ovulation factor appear to depend on some relationship of the pituitary gonadal mechanism but certainly no uniformity of reaction exists which will permit the formulation of a working hypothesis. One considers first that the method of quantitating the treatment is very crude; no assay of the potency of any given gland, and no idea of the relative rates of absorption are possible.

The increased potency of the anterior lobe of gonadectomized animals when implanted [Engle (26), Evans and Simpson (27)] shows clearly the existence, although not the nature of a reciprocal relationship between these two glands.

In an abstract [Smith and Engle (28)] we stated that the hypophysis of the guinea-pig showed a considerable variation in transplant potency, depending on the stage of the cycle. This observation has been extended by Wolfe (29) to apply also to the hypophysis of the sow. The greatest potency was found to exist in the hypophyses of sows with small inactive corpora and large follicles of 6 to 8 mm. in diameter, that is, during the progressive phase of follicular development.

Loewe (30) and Frank, Frank, Gustavson and Weyerts (31) first reported the presence of the female sex hormone in the circulating blood of normal women. Later, Frank and Goldberger (32) reported that an abrupt increase in the amount of the hormone took place, beginning with the 10th to 15th days of the cycle and rose with increasing concentration until the first day of menstruation. It is difficult to apply this datum to the pituitary-gonadal relationship, save to comment that this hormone appears to be at its lowest concentration during the period of follicular growth and maturation of the ovum when it must be assumed that the anterior lobe is acting directly on the follicle. If such an assumption can be made, then this condition is similar to that believed to occur in the guinea-pig and the sow, when the greatest activity occurs during the phase of follicular growth.

Burch and Cunningham (33) reported that the injection of commercial placental extract into adult castrated female rats tends to increase

the gonad-stimulating capacity of the anterior lobe of the recipient. Meyer, Leonard, Hisaw and Martin (34) found that the hypophyses of similarly treated animals had a lessened activity as determined by the weight of ovaries of immature host rats. Later, Leonard, Meyer and Hisaw (35) injected young rats (both pre- and post-pubertal animals being used) with relatively large doses of Amniotin-Squibb. Their conclusions are that the "injection of follicular hormone prevents the ovaries of immature rats from reaching full growth and development, as indicated by the weight of these glands." This was thought to be due to the effect of the oestrin on the hypophysis.

Previous to this report of Leonard, Meyer and Hisaw, Moore and Price (36) and Spencer, Gustavson and D'Amour (37) reported that the ovaries and testes of animals injected with oestrin were less in weight than those of the controls. These data are all obtained from animals receiving dosages (20 R.U. daily) much greater than any physiological condition existing in normal animals. The latter investigators being more interested in observing changes in growth than in gonad condition following oestrin treatment, reported that the oestrin treated animals gained weight less rapidly than the controls. It should be observed, however, that the oestrin injected animals would probably show a greater spontaneous activity (*vide* Slonaker) as a result of the prolonged oestrin treatment, and might be expected to show a lesser amount of body fat. The crucial observations on body length, tail length and length of the long bones were not made by the latter group. In the absence of these data it is felt that data on body weight of injected and control groups are not adequate to determine the effects of any hormonal imbalance between the female sex hormone and the anterior pituitary growth factor.

A study of the growth of the ovarian follicle in pre-pubertal mice [Engle (38)] gives some indication of the physiological relationship between the ovary and the anterior lobe in the normally developing mouse.

By measuring the inter-thecal diameters of the 10 largest follicles in each ovary of 3 mice for each day of life between birth and puberty, a curve of the growth of the follicle was obtained. It was noted that there were two phases, the first from birth to the 12th day, the second from the 12th day to puberty at about the 37th day. On the 12th day the first appearance of the antrum folliculi is seen. On the 13th and 14th this is augmented slightly, while on the 15th day the antral cavity is greatly increased, almost to the average size of the follicle at first oestrus. From this 15th day to first oestrus the growth of the follicle has quantitatively no important growth change. What changes do occur are those of tissue differentiation.

This period around the 15th day appears to be a crucial point in the life of the prepubertal mouse (MacDowell, Gates and MacDowell) in which other changes occur in the organism as significant as this follicular change.

The speculative question remains as to why the follicle, which we assume to be dependent for its growth on the gonad-stimulating factor of the anterior pituitary, should show such markedly accelerated growth in so short a time (two days) after the first appearance of the antrum and the primary liquor folliculi.

It is not assuming too much to consider that some such growth and elaboration of the follicular hormone are essential to stimulate the secretion or the liberation of the stimulating factor of the anterior lobe. One may suggest this in the face of the evidence regarding the depressing action of oestrin on the gonad-stimulating capacity because in the latter instances we are dealing with artificially introduced dosages of massive strength, while in the former we are observing a physiologically graded relationship.

These studies on the ovarian follicle in the 20 days immediately preceding oestrus, indicate that these large follicles must be secreting—and liberating—certain amounts of oestrin from the 15th day of life, and again emphasize the fact that puberty is a process of slow development, in which the first oestrus or first menstruation is merely an incident, expressive of a cumulative action of a delicate hormone balance in the organism. Since the follicles have attained nearly their full size in less than half the life span before puberty, it is clear that the changes occurring, which cannot be morphologically detected, must be those of slow physiological maturation of the organs which, operating satisfactorily together, constitute the living animal.

While the factors involved in this progressive development of puberty are immediate expressions of the pituitary-gonadal complex, other indirect or masked factors operate. Under the general head of environmental factors are grouped the extra-somatic influences, and are probably related to the genital system and problem of puberty only as the somatic conditions are so related. In our report on maturity in normal mice, it was pointed out that the earlier maturation from our series compared with those of Parkes (39) might be due to environmental as well as genetic factors. These data were collected from a colony maintained under good conditions in California. The animals studied in the present report are taken from a different stock, purchased three years ago from a local dealer, and maintained under quite similar dietary and hygienic conditions as were the previously reported California animals, but in New York City. It is of interest to note that the range of establishment of the vaginal orifice in this stock, after having been maintained under the same conditions of care and food, but quite different climate, is of the same nature as in the stock of the former colony. In each colony, however, there has been a deliberate selection of females for breeding stock which had produced large and healthy litters. It is possible that selection of this good breeding stock also resulted in the selection of an early maturing strain.

In their recent paper on this subject, Mirskaia and Crew report a definite genetic factor in maturity, with two distinct strains. Since they state that the strain which is distinctly slower in developing was purchased from a dealer, it is essential to demonstrate that the same differences exist in the two strains after several generations of the newly introduced group have been raised under the same conditions as the early maturing line. Long and Evans have felt that "a superior nutrition need not hasten the establishment of ovarian function." Evans and Bishop (3), however, state that oestrus occurs within a day or two of the rupture of the vaginal plate, "except in those cases of a suspected malnutrition." In regard to this one factor it is well known that the vaginal plate eventually ruptures in animals which have been spayed early in life.

In their investigation of the factor of the amount of mother's milk in the rate of growth of mice, MacDowell, Gates and MacDowell (40) were able to produce animals which at 15 days were as heavy as normal animals which at twice the age were just beginning to show the first signs of puberty. While these authors were primarily concerned with the growth response, records were kept regarding pubescence in the animals with this exceptionally rapid rate of growth. In a private communication Dr. E. C. MacDowell has been kind enough to inform me, and permit me to say, that the age of establishment of the vaginal orifice is distinctly below that of any mice save those which have had specific hormone treatment, and 7 of 13 showed an oestrus smear before the 25th day of life. Parkes (41) transferred litters of mice to rat foster mothers, and obtained a distinctly greater body weight than the norm for mice at each age day, but says nothing regarding age of puberty.

It is evident that while the interaction of the gonads and the anterior lobe affords the physiological mechanism for the first cycle as for the rhythmically succeeding ones, it is evident that this in turn is influenced by somatic factors, and that a general somatic maturity, as well as genital maturity, is essential to a complete reproductive potential.

SUMMARY

1. Immature mice, when implanted with anterior pituitary, exhibit certain phases of puberty. Of the 63 mice the vaginal plate ruptured on the third or fourth day after the first treatment. First induced oestrus followed in from 1 to 5 days after appearance of the vaginal orifice in all of them. Thirty-seven of the 63 mated during this first oestrus. Thirty of these 37 were killed and it was found that ovulation had occurred in 11. The remaining 7 of the animals which had mated did not show the pregnancy sign.

2. Thirty-three of these treated mice, including the 7 which had mated but were not sacrificed, were maintained with males, and records were made of the succeeding oestrus.

3. This oestrus, the first normal oestrus, occurred at a significantly later date than the first oestrus for normal mice, as also the first pregnancy. It is possible that the induction of the first oestrus delayed the appearance of the normal cycle.

4. The bearing of these observations on the concept expressed by the term, "precocious sexual maturity," is discussed.

BIBLIOGRAPHY

1. Long, J. A. and H. M. Evans: The oestrous cycle in the rat and its associated phenomena. Mem. Univ. of Calif. **6:** 5. 1922.
2. Mirskaia, L. and F. A. E. Crew: Maturity in the female mouse. Proc. Roy. Soc., Edinburgh, **50:** 179. 1930.
3. Evans, H. M. and K. S. Bishop: On the relations between fertility and nutrition. J. Metab. Research, **1:** 1. 1922.
4. Stone, C. P.: The family resemblance of female rats with respect to (1) the ages of first oestrus and (2) the body-weights. Am. J. Physiol. **77:** 625. 1926.
5. Engle, E. T. and J. Rosasco: The age of the albino mouse at normal sexual maturity. Anat. Rec. **36:** 383. 1927.
6. MacDowell, E. C. and E. M. Lord: Reproduction in alcoholic mice. Arch. f. Entwcklngsmech. d. Organ, **109:** 549. 1927.
7. Lipschütz, A.: Das Gesetz der Pubertät. Deutsche med. Wchnschr. **53:** 1079. 1927.
8. Lipschütz, A.: On some fundamental laws of ovarian dynamics. Biol. Rev. **2:** 263. 1927.
9. Lipschütz, A.: New developments in ovarian dynamics and the law of follicular constancy, **5:** 283. 1928.
10. Allen, E. and E. A. Doisy: The induction of a sexually mature condition in immature females by injection of the ovarian follicular hormone. Am. J. Physiol. **69:** 577. 1924.
11. Smith, P. E.: Hastening development of female genital system by daily homoplastic pituitary transplants. Proc. Soc. Exper. Biol. & Med. **24:** 131. 1926.
12. Smith, P. E.: The induction of precocious sexual maturity by pituitary homeotransplants. Am. J. Physiol. **80:** 114. 1927.
13. Smith, P. E. and E. T. Engle: Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. Am. J. Anat. **40:** 159. 1927.
14. Zondek, B. and B. Aschheim: Das Hormone des Hypophysenvorderlappens. Klin. Wchnschr. **6:** 248. 1927.
15. Siegmund: Ueber den Einfluz des Hypophysenvorderlappens auf den Ablauf der Sexualfunktion. Zentralbl. f. Gynäk. **52:** 1189. 1928.
16. Ehrhardt: Hypophysenvorderlappen und Genitale (Tierexperimentelle Untersuchungen). München. med. Wchnschr. **75:** 785. 1928.
17. Seitz, L.: Arch. f. Gynäk. **137:** 584. 1929.
18. Engle, E. T.: Pregnancy following super-ovulation in the mouse. Proc. Soc. Exper. Biol. & Med. **25:** 83. 1927.
19. Mirskaia, L. and F. A. E. Crew: On genital maturity in an immature soma. J. Exper. Biol. **7:** 248. 1930.
20. Engle, E. T.: Ovarian responses. J. A. M. A. **93:** 276. 1929.
21. Frank, R. T., H. M. Kingery and R. G. Gustavson: The female sex hormone. J. A. M. A. **85:** 1558. 1925.
22. Frank, R. T.: The female sex hormone. 1929. Charles C. Thomas, Springfield, Ill.
23. Friedman, M. H.: Mechanism of ovulation in the rabbit. II. Ovulation produced by the injection of urine from pregnant women. Am. J. Physiol. **90:** 617. 1929.
24. Shelesnyak, M. C.: The production of placentomata in young rats following gonadal stimulation with pituitary implants. Am. J. Physiol. 1931. (In press.)
25. Wiesner, B. P. and L. Mirskaia: On the endocrine basis of mating in the mouse. J. Exper. Physiol. **20:** 273. 1930.
26. Engle, E. T.: The effect of daily transplants of the anterior lobe from gonadectomized rats on immature test animals. Am. J. Physiol. **88:** 101. 1929.
27. Evans, H. M. and M. E. Simpson: A comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate the ovary. Am. J. Physiol. **89:** 371. 1929.
28. Smith, P. E. and E. T. Engle: Evidence of a correlation between the amount of gonadal-stimulating hormone present in the pituitary of the guinea-pig and the stage of the reproductive cycle. Anat. Rec. **42:** 38. 1929.
29. Wolfe, J. M.: A quantitative study of ovulation in the rabbit. Proc. Soc. Exper. Biol. & Med. **28:** 318. 1930.
30. Loewe, S.: Nachweis Brunsterzeugender Stoffe im weiblichen Blute. Klin. Wchnschr. **45:** 1407. 1925.

31. Frank, R. T., M. L. Frank, R. G. Gustavson and W. W. Weyerts: Demonstration of the female sex hormone in the circulating blood. I. Preliminary report. J. A. M. A. **85**: 510. 1925.

32. Frank, R. T. and M. A. Goldberger: The female sex hormone. IV. Its occurrence in the circulating and menstrual blood of the human female: Preliminary report. J. A. M. A. **86**: 1686. 1925.

33. Burch, J. C. and R. S. Cunningham: Effect of placental extracts on ovarian stimulating properties of anterior hypophysis. Proc. Soc. Exper. Biol. & Med. **27**: 331. 1930.

34. Meyer, R. K., S. L. Leonard, F. L. Hisaw and S. J. Martin: Effect of oestrin on gonad stimulating power of the hypophysis. Proc. Soc. Exper. Biol. & Med. **27**: 702. 1930.

35. Leonard, S. L., R. K. Meyer and F. L. Hisaw: The effect of oestrin on development of the ovary in immature female rats. This Journal, **15**: 17. 1931.

36. Moore, C. R. and D. Price: The question of sex hormone antagonism. Proc. Soc. Exper. Biol. & Med. **28**: 38. 1930.

37. Spencer, J., R. G. Gustavson and F. E. D'Amour: Effect of estrin injections on the growth curve of young rats. Proc. Soc. Exper. Biol. & Med. **28**: 500. 1931.

38. Engle, E. T.: Prepubertal growth of the ovarian follicle in the albino mouse. Anat. Rec. **48**: 341. 1931.

39. Parkes, A. S.: The age of attainment of sexual maturity in the albino mouse. J. Roy. Micr. Soc. 315. 1925.

40. MacDowell, E. C., W. H. Gates and C. G. MacDowell: The influence of the quantity of nutrition upon the growth of the suckling mouse. J. Gen. Physiol. **13**: 529. 1930.

41. Parkes, A. S.: Note on the growth of young mice suckled by rats. Ann. Applied Biol. **16**: 171. 1929.

THE REGULATION OF THE HYPOPHYSIS BY THE TESTICLE, AND SOME PROBLEMS OF SEXUAL DYNAMICS

(EXPERIMENTS WITH PARABIOTIC RATS)

THALES MARTINS AND ARNOLDO ROCHA
From the Laboratory of Endocrinology, Oswaldo Cruz Institute
RIO DE JANEIRO, BRAZIL

The parabiotic experiments on a normal adult female rat connected with a castrated partner, described by Matsuyama and Yatsu, brought into evidence very important facts. The ovaries and uterus of the former presented a large hypertrophy, at times pyometra or hydrometra; enormous cystic ovarian follicles, or considerable luteinization were found. In cases of parabiosis of two normal females no noteworthy alteration was observed.

Goto reproduced and confirmed the above experiments.

Matsuyama did not draw any causal conclusion therefrom, and Goto admitted the existence of a "Kastrohormon," a substance which would be present, in a greater quantity, in castrated animals, without any further analysis as to its nature.

Our present knowledge, mainly due to the works of Evans, Zondek and Aschheim, Smith and Engle, on the influence of the anterior hypophysis upon the gonads enables us to understand the phenomena above referred to: the prehypophyseal hormones which exist in greater quantity in the circulation of castrated animals are those which by their passage into a normal partner induce such effects to appear. In other words, the parabiosis of a normal animal with a castrated one ("C.M." or "S.F.")* is equivalent to a chronic treatment with hormones of the anterior lobe.

The problem was taken in hand with such an orientation by Kallas (at Lipschütz Institute) by Fells and Martins (2, 5, 7).

Kallas (1) brought into evidence the characteristic effects of the anterior hypophysis: the induction of precocious puberty.

By means of parabiosis of a normal infantile rat with an infantile castrated one, the former after a few days exhibits signs of precocious sexual maturity. By means of injection of blood from a castrated infantile animal, a similar phenomenon is induced in infantile rats or mice (Martins, 2).

The ovarian hormone also passes from the normal female into the spayed one; prolonged oestrous stages can be induced in the latter, if the vascular connection is sufficiently ample.

The symptomatology found in parabiosis of infantile females with castrated ones is the same as that of hypophyseal implantations. From

*"C.M."—castrated male; "S.F."—spayed female.
"N.M."—normal male; "N.F."—normal female.

the sixth or seventh day after their junction the animals already manifest the oestrous type in the vaginal smear; the ovarian follicles become mature; in many cases, the formation of corpora lutea is observed and even ovulation. When the parabiotic life lasts for a longer time, monstrous appearances can be observed, exactly as they are seen in animals which have been treated for a long time with implantations of hypophysis, as shown in Fig. 1.

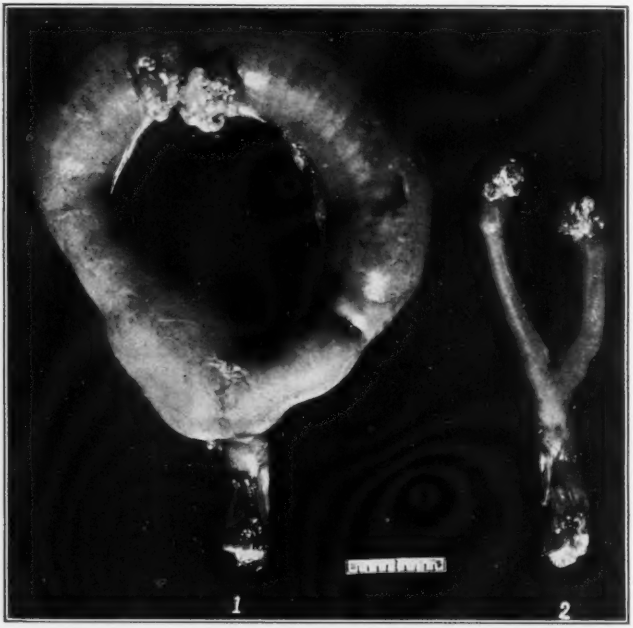


Fig. 1
1. Genitals of a female rat, 110th day after parabiotic union with a castrated male.
2. Genitals of the solitary normal control.

Just as the implantation of hypophysis has the property of inducing precocious sexual maturity, be the donor N.M. or N.F., in parabiosis the sex of the castrated partner is also of no consequence as to its characteristic effects. Nevertheless, Martins (4) has observed a predominance of follicular effect in the partner's ovaries, *i. e.*, large follicles were detected mainly, when the other partner was a C.M.; while in the case of S.F. the predominant type is that of luteinization.

It is not only the female which can be used to test the prehypophyseal hormones circulating in castrated animals. In the case of joined infantile C.M. + N.M. or S.F. + N.M. the normal male also presents symptoms of pubertas praecox, characterized by increase of the testicles and, principally, of the accessory glands; the time required is longer than in the case of a female, 10 to 15 days (Kallas) (exactly as in case of hypophyseal

implantations; the treatment of the N.M. must be more protracted than that of the N.F.).

But, while in the twinned N.F. + S.F. the ovarian hormone secreted to excess can pass into the S.F. and induce oestrous cycles, in the case of parabiosis of N.M. + C.M. no sign of testicular hormoneal influence on the C.M. is detectable, even after a parabiotic life lasting several months (Martins, 5). Consequently, the testicular hormone does not pass from N.M. into C.M. in sufficient amount. Even in some couples of parabiotic life lasting 210 days, we did not succeed in observing any influence on the sequellae of castration; in the normal fellow, we noted a clear hypertrophic reaction of the interstitial elements.

Such increase of hypophyseal hormones has been verified even in women (Zondek, Fluhmann); the urine of ovariectomized women often shows a positive reaction with respect to ovaries of infantile animals. By injecting urine from castrated albino rats we never succeeded in obtaining a frank positive reaction.

The hypophysis of castrated animals, when implantated into infantile animals, manifests a greater activity than that of normal donors (Engle, Evans and Simpson).

Moreover, Martins' (8) experiments have shown that in cryptorchidic animals there is an increase of circulating prehypophyseal hormones. In the case of parabiotic N.M. + N.F. there is no alteration of the sexual phenomena in the latter or sometimes absence of oestrous cycles. In pairs of cryptorchid N.M. + N.F., there are protracted full-estrus stages (an *oestrus* lasting for even *42 successive days* has been observed), and increase of the ovary, although not so pronounced as in the case of junction with a castrated animal. In these experiments we took into consideration only those in which no antimasculine influence was observed on males; otherwise, one might think of a "hormoneal castration," *i. e.*, a testicular degeneration provoked by feminine hormones. This harmonizes with the results achieved by Evans and Simpson who, by the method of implantations, have shown that the hypophysis of a cryptorchid animal presents an average activity higher than that of a normal one, and a lower degree than that of a castrated one.

After ablation of the gonads, the organism being flooded by hypophyseal hormones, two interpretations would be possible: (1) the hormones might accumulate in blood owing to lack of the fixing organs; (2) there might mainly be a hypophyseal hyperfunction owing to lack of regulating organs, *i. e.*, the gonads, which might exert a certain inhibition on the pituitary gland.

This second possibility was admitted, from the beginning, by Kallas and Martins, and is now established by decisive experiments. So the injection of extracts containing oestrine into the spayed partner, prevented the precocious sexual maturity of the normal one, but in the case of C.M. +

N.F. the treatment was inefficient, *i. e.*, the N.F. entered into puberty just as in the case of pairs which might have not been treated (Kallas, 2).

In other words, the feminine hormone seems to have an inhibiting activity on the female hypophysis, but less effective on that of the male. A prolonged treatment of females with oestrine retards the development of the ovaries [Del Castillo and Calatroni, at Houssay's Institute, in rats; Lipschütz (2) in cats], possibly as a result of an inhibition of the hypophysis. Moore and Price, Meyer, Leonard, Hisaw and Martin assume also that the gonadal hormones have an influence on the pituitary body.

The present work, upon which we already published a preliminary report (3) deals with the influence of the testicular hormone on the pituitary gland; it shows that there is a testicular hormone which regulates the hypophysis, and that this regulation within certain quantitative limits acts upon the hypophysis of the N.M. only. The results obtained with testicular implantations are here reported; the effects observed with various testicular extracts which have already given us positive results, will be dealt with in our next report.

METHODS

The parabiosis was performed following the technique initially published by Sauerbruch and Heyde (coelionastomosis). A lateral incision of the skin of the flank was made as long as possible, between shoulder and hip; the abdonimal cavity was also opened by an extensive incision of the muscular wall. The flaps of the muscular wound were then circularly sutured, and after that, in another stage, the cutaneous wound, always with catgut. In the first hours, a band of contentive adhesive plaster may be overlaid and the next day removed. The castration was made at the same operation.

The animals (albino rats) were litter mates, aged 19 to 30 days and weighing 16 to 30 grammes. Their food was always identical, being rich in vitamines.

The organ to be implanted was aseptically removed from the donor rat, then reduced to fine pulp and sometimes left at laboratory temperature (between 25° and 30° C.), for about 1 hour, in order to destroy its vitality, since we had in view implantations but not graftings.

The implantation was made by distributing the material through a tiny incision of the skin in two or three different spots in order that it might be better absorbed; with a fine pincer the skin was loosened making a kind of tunnel about two cms. in length, where the material was laid down. In the case of infantile donors (from 20 to 25 days of age), two testicles were always implanted at the same time. In the case of adult donors, the implanted quantity amounted to ½ or ⅓ of the organ, or about 0.20 grammes in weight. The treatment was initiated on the same day of the operation, being repeated every two or three days. A less intensive treatment proved to be little efficient.

TABLE 1

PARABIOSIS OF CASTRATED + NORMAL INFANTILE RATS; EFFECTS OF IMPLANTATIONS OF TESTIS AND OTHER ORGANS INTO THE CASTRATED PARTNER

Designation of Pair	Type	Initial Age, Days	Individual Weights, Grams	Treatment	Vaginal Smear of the Normal Partner	Autopsy — Days After Parabiotic Union	Weight of Pair	Autopsy Data.	Genitalia of the Normal Partner	
								Uterus	Ovaries	Vagina
A4	CM+NF	28	26	5 impl. inf. test.	Diestrus	14	85	Infantile	Initial follicular growth.	Initial stratification
A6	" "	22	21	5 impl. inf. test.	Diestrus	12	77	Infantile	Infantile	Infantile
A7	" "	23	21	5 impl. inf. test.	Diestrus	12	..	Infantile	Infantile	Infantile
A10	" "	21	26	4 impl. inf. test.	Diestrus	10	83	Infantile	Initial follicular growth.	Infantile
A16	" "	28	30	4 impl. inf. test.	8th day—Proestrus	9	90	Distended by secretion	Ripe follicles	Estrus
A17	" "	19	22	5 impl. inf. test.	Diestrus	9	68	Infantile	Medium sized follicles.	Initial stratification
A18	" "	..	23	4 impl. inf. test.	Diestrus	8	62	Infantile	Infantile	Initial stratification.
A19	" "	23	26	5 impl. inf. test.	8th day—Proestrus	9	78	Distended by secretion	Ripe folicles	Estrus
G31	" "	23	21	4 impl. adult test.	Diestrus	9	65	Infantile	Medium sized follicles.	Initial stratification
D45	" "	21	25	4 impl. adult test.	Diestrus	7	..	Infantile	Infantile	Infantile
D46	" "	21	25	5 impl. adult test.	Diestrus	10	84	Infantile	Infantile	Infantile
M90	" "	24	18	11 inject. test. Mac.	Diestrus	11	57	Infantile	Infantile	Infantile
A1	" "	31	27	11 inject. test. Mac.	Diestrus	11	68	Infantile	Infantile	Infantile
G37	" "	..	18	None	7th day—Estrus	8	54	Engorged by secretion	Ripe and atretic follicles	Estrus
M97K	" "	21	19	None	8th day—Estrus	11	67	Hyperemic	Ripe follicles	Estrus
G30	" "	23	31	4 impl. thymus	7th day—Estrus	9	75	Large, distended	Numerous ripe follicles, some atretic follicles	Estrus
G36	" "	..	16	6 impl. suprar.	11th day—Proestrus	12	56	Engorged by secretion	Medium sized follicles	Proestrus
D47	" "	30	22	4 impl. thymus	8th day—Estrus	9	66	Thickening of the muscular layer	Numerous ripe follicles; ova in the tube	Estrus
A5	SF+NF	28	24	5 impl. inf. test.	8th day—Estrus	14	77	Large, distended	Ripe follicles, abundant luteinisation	Estrus
A8	" "	22	26	5 impl. inf. test.	8th day—Estrus	12	..	Large, hyperemic	Numerous corpora lutea	Mataestrus
A20	" "	23	26	4 impl. inf. test.	8th day—Diestrus	9	68	Hyperemic	Large follicles, numerous atretic follicles	Proestrus
A21	" "	..	29	3 impl. inf. test.	6th day—Estrus	8	83	Distended by secretion	Ripe follicles	Estrus
A22	" "	..	24	3 impl. inf. test.	5th day—Estrus	7	69	Large, hyperemic	Ripe follicles; ova in the tube	Estrus
G34	" "	..	23	4 impl. adult test.	7th day—Estrus	9	75	Large, hyperemic	Ripe follicles; ova in the tube, numerous corpora lutea	Estrus
G35	" "	..	24	3 impl. adult test.	7th day—Estrus	9	77	Thickening of the muscular layer	Ripe follicles, abundant luteinisation	Estrus
D52	" "	28	25	5 impl. adult test.	6th day—Estrus	10	73	Thickening of the muscular layer	Ripe follicles, corpora lutea	Estrus
G32	" "	23	21	6 impl. suprar.	8th day—Estrus	12	78	Large, hyperemic	Ripe follicles, numerous atretic follicles	Estrus

C.M., castrated male; N.M., normal male; S.F., spayed female; N.F., normal female. The genitals of the castrated partners were found at autopsy and in section in every case to be infantile.

The implantations of other organs were made by the same technique.

In the case of injection with macerated material the organ was reduced to fine pulp, left for maceration in saline solution for a few hours; it was afterwards centrifugued and the supernatant liquid was injected once or twice a day.

The vaginal smear was examined every day, by the usual technique.

The material, removed by autopsy for histological observations, was fixed by Zenker-formol and usually stained by haematoxylin-eosine.

RESULTS

As seen from Table 1, in the pairs C.M. + N.F., untreated or subjected to implantation of suprarenals or thymus, the vaginal smear exhibited, right from the seventh day, the sexual maturity of the female; and the findings at autopsy showed the development of the genitalia which is characteristic of puberty. On the other hand, in the pairs subjected to testicular treatment, the genitalia kept the infantile appearance closely similar to that of the solitary normal controls. In a total of 13 experiments, the treatment only failed in two, a fact which perhaps may be accounted for by a bad absorption of the material.

In the pairs S.F.+N.F., the implantations exerted no influence on the results; puberty presented itself at the term normally observed by Kallas and Martins in the pairs not subjected to any treatment. The implantation did not retard the appearance of the characteristic signs of precocious sexual maturity.

The results are summarized in Table 2.

TABLE 2

SUMMARY OF TABLE 1

Type of Experiments	Total of Experiments	Number of Cases Showing Sexual Maturity
CM+NF, castrated treated with testis	13	2
" " control implantations	5	5
SF+NF, spayed implanted with testis	8	8
" " spayed implanted with suprarenal	1	1

We regarded as positive signs of sexual maturity a clear increase of ovarian follicles and a vaginal structure at least corresponding to the proestrus. As the greatest importance was given to the histologic examination, the weight of the organs was left aside.

As regards the castrate male partners it is to be noted that, even in those subjected to testicular treatment, the genitalia remained at an infantile state; the seminal vesicles, macro- as well as micro-scopically, did not present any stimulation, maintaining the same atrophic appearance of the solitary castrated control.

THE STRUCTURE OF THE HYPOPHYSIS AFTER CASTRATION

The alterations of the anterior hypophysis after castration are well known since Fichera's publications. There is no complete accordance in the results described by different investigators, a fact which may be mainly

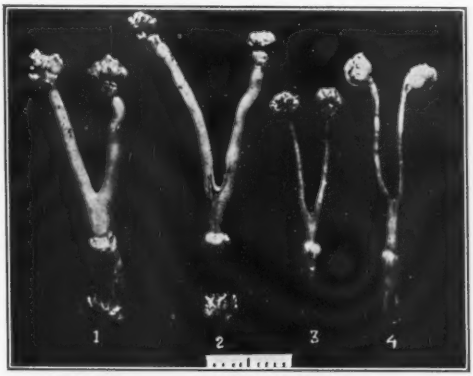


Fig. 2
Genitals of infantile female rats, in parabiosis with castrated partners:
1. Female united to a female implanted with testis (D52).
2. Female united to a male implanted with thymus (D47).
3. Female united to a male implanted with testis (D46).
4. Genitals of the female solitary control.
For further details, see the designation of pairs in Table 1.

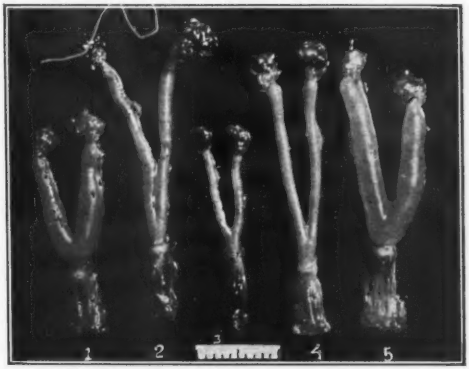


Fig. 3
Genitals of infantile female rats, in parabiosis with castrated partners:
1. Female united to a male, implanted with thymus (G30).
2. Female united to a female, implanted with testis (G35).
3. Female united to a male, implanted with testis (G31).
4. Female united to a female, implanted with testis (G34).
5. Female united to a male, untreated control (G37).

accounted for by the difference of reactions observed in distinct zoological species. In rats, Biedl-Zacherl encountered vacuolated cells, which were regarded as eosinophilic. Schleidt described the forms of signet-ring. Lehmann, Nukariya, Schenck, Van Wagenen and Addison, also published descriptions of those elements regarding them as derived from basophilic cells, an opinion which we also hold.

Lehmann implanted in adult rats gonads from donors of different species and verified that the histological normalization of the hypophysis was obtained only by means of implantation of gonads of the same sex as that of the recipient.

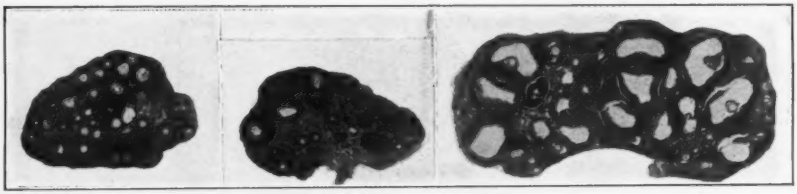


Fig. 4
Ovary of a female solitary control (age: 23 days), x18.

Fig. 5
Ovary of a female united to a castrated male, implanted with infantile testis (Pair A7), x18.

Fig. 6
Ovary of a female united to an untreated castrated male (Pair G37), x18.

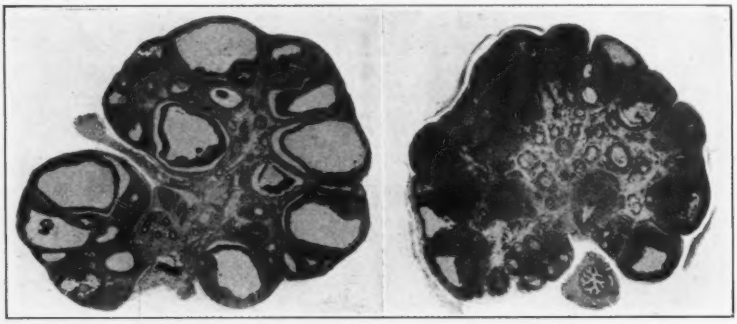


Fig. 7
Ovary of a female united to a castrated male, implanted with thymus (G30), x18.

Fig. 8
Ovary of a female, united to a spayed female, implanted with testis (G34), x18.

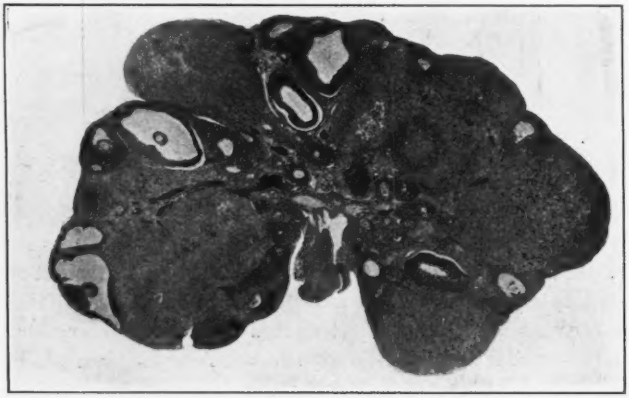


Fig. 9
Ovary of a female, united to a spayed female, implanted with testis (A5), x18.

In our experiments here reported, the hypophysis of the parabiotized was studied also. In the castrated partners, cells with incipient vacuolization appeared with relative abundance even after the lapse of only five or six days after castration (Fig. 15). The specific counting out of those elements showed that the testicular treatment had a sharp preventive influence on castrated males (Table 3). At the same time, the sexual condition of the normal female partner tested in the most proper manner the hypophyseal state of the castrated partner.

Fig. 10
Tubal ovum of a female united to a spayed female, implanted with testis (A22), x116.

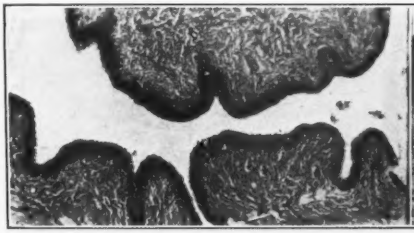


Fig. 11
Vagina of a female united to a castrated male, implanted with testis (A6), x100.

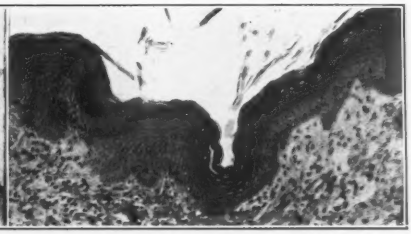


Fig. 12
Vagina of a female united to an untreated castrated male (G37), x100.

Fig. 13
Vagina of a female united to a spayed female, implanted with testis (A22), x100.

Fig. 14
Vagina of a female united to a castrated male, implanted with thymus (G30), x100.

Consequently, we have in the same experiment the morphological hypophysial test of the castrated, and the functional sexual test of the normal partner, as indicators of the activity of the anterior pituitary.

TABLE 3

EFFECTS OF THE TESTICULAR IMPLANTS ON THE HYPOPHYSIS AND GENITAL DEVELOPMENT OF PARABIOTIC RATS

Designation of Pair	Type	Material Implanted into the Castrated Partner	Autopsy—Days After Parabiotic Union	"Castration Cells" in the Hypophysis of the Castrated Partner	Genitals of the Normal Female Partner
A4	CM+NF	Infantile testis	14	2	Infantile
A6	" "	Infantile testis	12	0	Infantile
A7	" "	Infantile testis	12	3	Infantile
A16	" "	Infantile testis	9	0	Infantile
A17	" "	Infantile testis	9	0	Infantile
A19	" "	Infantile testis	9	0	Infantile
G31	" "	Adult testis	9	1	Infantile
D46	" "	Adult testis	10	4	Infantile
G30	" "	Thymus	9	24	Mature
D47	" "	Thymus	9	13	Mature
A5	SF+NF	Infantile testis	14	26	Mature
A8	" "	Infantile testis	12	31	Mature
A21	" "	Infantile testis	8	23	Mature
G34	" "	Adult testis	9	35	Mature
G35	" "	Adult testis	9	39	Mature
D52	" "	Adult testis	10	17	Mature
G32	" "	Suprarenal	12	15	Mature

The number of "castration cells" represents the total count in 10 microscopic fields, x1000.

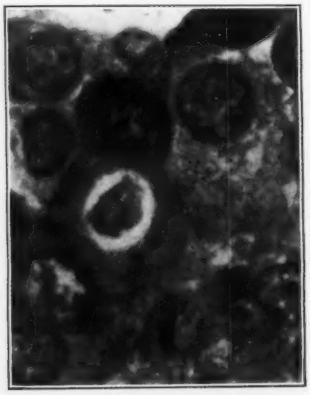


Fig. 15

Castration cell, with initial vacuolisation; anterior hypophysis of a female rat, 9th day after castration (G35), x2000.

DISCUSSION

The experimental findings reported above support the existence of a testicular hormone controlling the anterior hypophysis. The absence of that limiting factor accounts for the hyperfunction of the gland after gonadectomy. The activity of the implantations of infantile testicles brings into evidence the fact that the hormone referred to is already secreted by the infantile testicle.

Such pronounced activity, even in the prepuberal condition is, quite surprising, whilst the influence of the gonad exerted on the sexual accessory organs is still of so little evidence.

The fact that the testicular material, of identical quantities, acted only on the masculine hypophysis deserves to be emphasized. Consequently, a certain sexual specific condition might exist, at least within certain quantitative limits.

These results harmonize with those of Kallas (2) and Lehmann, obtained by another kind of experiments.

Moreover, this sexual difference of hypophysis reaction was also verified by Martins and Rocha (2) in gonadic grafts. Ovarian well vitalized grafts on male castrated rats had no preventing activity upon the appearance of the castration cells.

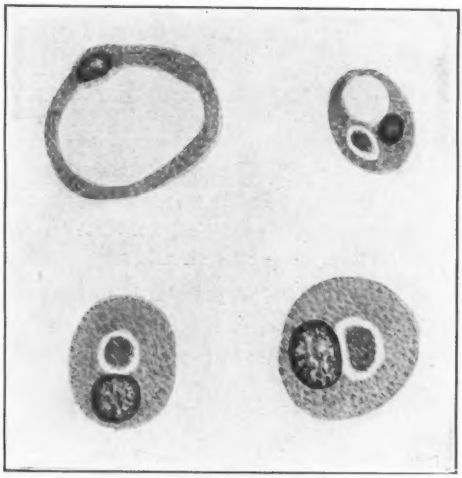


Fig. 16

Camera lucida drawing, x1400. Castration cells, showing initial vacuolisation, and signet-ring form. Anterior hypophysis of a female rat, 8 weeks after gonadectomy.

In spayed females grafted with testicle, an evident virilization took place, the clitoris being transformed in a penisoide organ; yet, the hypophysis presented all the alterations of castration.

The hyperfeminization of male guinea pigs (Steinach, Lipschütz, Sand, Athias, etc.) suggest some considerations as regards gonado-pituitary interrelationship. In order to account for the permanent development of the teats and absence of corpora lutea in the grafted ovaries, one has to take into consideration, apart from a functional difference of the masculine hypophysis, a greater richness in stimulating factor of the follicles, (Martins, 4), also the question of its regulation. In the present case, we are dealing with an ovary grafted into a male soma. As we have already seen, there cannot be the same equilibrium as in the cases of homologous

grafts; the Lipschütz theory of the Y-substances must be taken into consideration.

It is reasonable to conclude that the alterations of the pituitary body after castration indicate hyperfunction; if the castration cells are supposed to be of basophilic origin, this would support the hypothesis, mentioned by Evans and Simpson, that the basophilic cells are the secreting substratum of the gonad-stimulating hormones.

The alterations of the hypophysis of rats during pregnancy are of a different kind; should there also take place an increased secretion of the hormones referred to? It is difficult to grasp that the hypophysis in pregnancy should be responsible for the inundation of the pregnant woman's organism with gonad-stimulating hormones, while in this physiological condition there are enormous quantities of ovarian hormone. Thus this would mean the maximum of prehypophyseal function just when the maximum of inhibiting hormone is in circulation. The concurrence of other factors, as the placenta, must also be taken into consideration

As regards the secreting elements of the testicular hormone which regulates the hypophysis, we are led to believe that such elements proceed from the germinal epithelium. Thus, the alterations of the germinal epithelium induce in the hypophysis attenuated lesions of castration. Moreover, as it was shown by parabiotic experiments on cryptorchids, there is an increase of hypophyseal hormones circulating in the blood.

At first glance, it would be surprising that an infantile testicle is sufficient for a function, while that of an adult cryptorchidic is not. But, apart from certain morphological differences, we must take into consideration the fact that the infantile testicle has under its dependence an infantile hypophysis while the testicle of a cryptorchidic has to control an adult hypophysis.

If we compare the influence of the testicle on the hypophysis and on the accessory glands, in various experimental conditions, we find some dissociations of the effects.

First type: In cases of troubles of spermatogenesis (cryptorchidism, vasoligation, etc.), there are alterations of the hypophysis, but the accessory genitalia are normal.

Second type: In infantile or adult castrated, the testicular implantation normalizes the hypophysis, but does not exert the least influence on the accessory genitalia. Even intensive treatment with non-living testicular implants, beginning with the very day of castration, has no preventing action upon the atrophy of the accessory glands of rats and mice [Martins and Rocha, (1); Moore]. Yet, the accessory glands are a good test for testicular hormone [Moore and Gallagher, Martins and Rocha, (1); Loewe and Voss].

In the first type therefore, a hormonic level exists, which is sufficient

to keep the genitalia in normal condition, but it does not suffice for the same as regards the hypophysis.

In the second type, the conditions are reversed; the hormone introduced by treatment is sufficient for the hypophysis, but not so for the genitalia. If we take into consideration exclusively a quantitative criterion, we meet with a contradiction. In the former case, the threshold of the hypophysis would be higher than that of the genitalia, while in the latter it would present, on the contrary, a lower threshold.

By an intensive treatment of castrated males with testicular aqueous extracts we succeeded in keeping the hypophysis in normal condition, without inducing the slightest influence on the accessory glands, as we shall deal with more in detail, in our next report.

A qualitative consideration is, therefore, enjoined upon us, and this leads us to believe in the existence of more than one testicular hormone; the hormone which regulates the hypophysis would be different from that which regulates the accessory genitalia.

To conclude, the reciprocal pituitary-gonadic interrelation renders the interpretation of the mechanism of puberty and the sexual cycle still more complex.

In the female, either the sexual maturity or the cyclical variations of oestrus, would be dependent on the hypophyseal-gonadic equilibrium, and the cyclical morphological variations of the ovarian follicles bring into plain evidence the variations of hypophyseal regulation depending on ovarian hormones.

In the adult males which usually do not exhibit a sexual cycle, the hypophysis would be subjected to a permanent and stable regimen of regulation, compatible with a permanent level of sexual activity. The structure of the testicle supports this interpretation; even if the elaboration of the hormone which regulates the hypophysis would occur only at a determined stage of the germinal cells, in spite of the seminal cycle, the effects would be compensated by the simultaneous existence of abundant elements which represents all the different stages of the spermatogenic cycle.

SUMMARY

We have experimentally confirmed that the parabiosis of an infantile female rat with an infantile castrated one induces a precocious sexual maturity of the former, a few days after their junction. This phenomenon is due to the passage of the prehypophyseal hormones from the castrated into the normal rat. In the pairs in which the castrated partner is a male, testicular implantations or injections of mascerated testicle prevent the precocious sexual maturity of the female partner. Consequently, there exists a testicular hormone which regulates, directly or indirectly, the anterior hypophysis; when this hormone is absent, the hypophysis enters into hyperfunction.

In the pairs, spayed female plus normal female, the mentioned treat-

ment in identical doses does not prevent the precocious sexual maturity of the normal animal. Consequently, the hypophysis of the male is at least more sensitive towards the testicular hormone than that of the female.

The infantile testicle already secretes the hormone which regulates the hypophysis. The histological alterations of the hypophysis after castration are prevented or disappear in castrated males after testicular implants or injections.

In castrated males one and the same treatment can be efficient upon the hypophysis, and inefficient to correct the atrophy of the accessory genitalia. The hormone regulating the hypophysis is probably secreted by the germinal epithelium. The identity of this hormone with that which regulates the development of the accessory genitalia is open to doubt.

BIBLIOGRAPHY

Addison, W: Anat. Rec. **10:** 171. 1916.
Athias, M.: Compt. rend. Soc. de biol. **78:** 410. 1915.
Biedl, A: Innere Sekretion, **2:** 108. 1916.
Del Castillo, E. and C. Calatroni: Rev. Soc. Argent. de biol. **6:** 108. 1930.
Engle, E.: Am. J. Physiol. **88:** 101. 1929.
Evans, H. and M. Simpson:
(1) Am. J. Physiol. **89:** 379. 1929.
(2) J. A. M. A. **91:** 1337. 1928.
Fels, E.: Arch. f. Gynäk. **138:** 16. 1929.
Fichera: Arch. ital. di biol. **43:** 405. 1905.
Fluhmann, C.: Am. J. Obst. & Gynec. **20:** 1. 1930.
Goto: Arch. f. exper. Path. u. Pharmakol. **94:** 124. 1922.
Heyde: Ergebn. d. Physiol. **12:** 564. 1912.
Houssay, B. A. and L. Giusti: Rev. Soc. Argent. de biol. **5:** 47. 1929.
Kallas, H.:
(1) Arch. f. d. ges. Physiol. **223:** 232. 1929.
(2) Klin. Wchnschr. **9:** 1345. 1930.
Lehmann, J.: Arch. f. d. ges. Physiol. **216:** 729. 1927.
(1) Akadem. Anzeiger. No. 20. 1929.
Loewe, S. and H. Voss:
(1) Akadem. Anzeiger. No. 20. 1929.
(2) Klin. Wchnschr. **9:** 481. 1930.
Loewe, S. and H. Voss: (2) Klin. Wchnschr. **9:** 481. 1930.
Lipschütz, A.:
(1) Arch. f. d. ges. Physiol. **207:** 548. 1925.
(2) Arch. internat. de pharmacodyn. et de thérapie, **38:** 57. 1930.
Martins, Th.:
(1) Endokrinologie, **7:** 180. 1930.
(2) Suppl. Mem. Inst. Oswaldo Cruz, **10:** 233. 1929.
(3) Brasil-med. **44:** 906. 1930.
(4) Compt. rend. Soc. de biol. **105:** 99. 1930.
(5) Compt. rend Soc. de biol. **103:** 1341. 1929.
(6) Suppl. Mem. Inst. Oswaldo Cruz, **12:** 301. 1929.
(7) Compt. rend. Soc. de biol. **102:** 605 a.614. 1929.
(8) Compt. rend Soc. de biol. **105:** 789. 1930.
Martins, Th. and A. Rocha:
(1) Compt. rend. Soc. de biol. **102:** 480; 485. 1929.
(2) Compt. rend. Soc. de biol. **105:** 793. 1930.
(3) Compt. rend. Soc. de biol. **105:** 795. 1930.
Matsuyama, R.: Frankfurt. Ztschr. f. Path. **25:** 432. 1921.
Meyer, R., S. Leonard, F. Hisaw and S. Martin: Proc. Soc. Exper. Biol. & Med. **27:** 702. 1930.
Moore, C., and T. Gallagher: Am. J. Physiol. **89:** 388. 1929.
Moore, C., and D. Price: Proc. Soc. Exper. Biol. & Med. **28:** 38. 1930.
Moore, C.: J. A. M. A. **94:** 1912. 1930.
Nukariya, S.: Arch. f. d. ges. Physiol. **214:** 697. 1926.
Riddle, O.: Physiol. Rev. **11:** 63. 1931.
Sand, K: J. de Physiol. et de path. gén. **19:** 305. 1921.
Schenk, F.: Ztschr. f. Geburtsh. u. Gynäk. **91:** 483. 1927.
Schleidt: Zentralbl. f. Physiol. **27:** 1170. 1914.
Smith, P. and E. T. Engle: Am. J. Anat. **40:** 159. 1927.
Steinach, E.: Arch. f. d. ges. Physiol. **144:** 71. 1912.
Van Wagenen, G.: Anat. Rec. **29:** 398. 1925.
Yatsu, N.: Anat. Rec. **21:** 217. 1921.
Zondek, B.: Klin. Wchnschr. **9:** 303. 1930.
Zondek, B. and S. Aschheim: Klin. Wschnschr. **6:** 248. 1927.

CEREBRAL ADIPOSITY WITH MENTAL DEFICIENCY AND RETINITIS PIGMENTOSA: THE LAURENCE-BIEDL SYNDROME

EDWARD WEISS, M.D.
From the Department of Medicine,* Jefferson Medical College Hospital
PHILADELPHIA

In 1925 Solis-Cohen and Weiss (1) reported four children of one family who exhibited marked adiposity with mental deficiency, genital dystrophy and diminution of vision due to retinitis pigmentosa. Another anomaly, polydactylism, was present in two. The fat distribution was of the Fröhlich type. Roentgen-ray examinations of the sella region did not show conclusive changes nor in any other way could a pituitary lesion be proved. Four unaffected children in the same family were entirely normal.

Search of the literature revealed a remarkably similar family group reported by Laurence and Moon (2) in 1866. Four of eight brothers and sisters of healthy parents showed retinitis pigmentosa, adiposity, genital dystrophy and mental deficiency. Three of these likewise showed imperfections of bony and muscular development leading to defective gait. There was no signs of rickets, of scrofula nor of syphilis. The authors recognized that the retinal changes were but part of a general developmental failure and that especially in view of the mental condition these persons could be, not inaptly, compared to cretins.

Similar cases were recognized as a syndrome by Biedl (3) in 1922. He discussed the condition as a distinct one and characterized it as consisting of obesity (of cerebral rather than pituitary origin), genital hypoplasia, atrophy of the optic nerve with pigment degeneration of the retina (frequently of an atypical form), hemeralopia or night-blindness, polydactylism and mental deficiency. He presented two cases with this clinical picture in a brother and sister.

Because of the original description by Laurence and Moon and the elaboration as a distinct cerebral obesity syndrome by Biedl the name Laurence-Biedl syndrome was suggested for the condition.

In 1929 Ricaldoni and Isola (4) described four cases in one family, calling the condition Bardet's syndrome. Bardet's thesis (5), published in 1920, was entitled "A Syndrome of Infantile Obesity with Polydactylism and Retinitis Pigmentosa." He reported a case in a girl of 11 and arrived at the conclusion that it was a true syndrome with a distinct individuality due to a lesion attacking the pituitary gland during the period of foetal development. Many cases of the fully developed syndrome have been reported (the total is now 45), a complete bibliography of which is included in a recent excellent article by Ornsteen (6), who added 3 cases

*Service of Dr. Thomas McCrae.

in a contribution to the pathogenesis of the syndrome. I wish now to report another case.*

The patient, a white girl of Polish parentage, aged 15 years, made no particular complaint herself. She was sent by her physician (Dr. S. C. Mazaleski) and brought by her uncle, who told us that she had some difficulty with vision and was deficient mentally.

Family History. Her parents were living. There was no history of adiposity or eye trouble in any other member of the family. We were able to study the father's condition as he came to see the patient after she had been some time in the hospital. He gave the impression of being rather below the average in

Fig. 1. Photograph of the patient at the age of 4 years, before the acute illness which brought to light the congenital disorder.

mental powers and he had a good deal of arteriosclerosis and some myocardial change. He showed no evidence of syphilis and the serological tests were negative. About three months later he committed suicide. The mother and one brother were said to be healthy and normal.

Past History. The patient had had measles in early childhood and was regarded as a normal child up to the age of four years, at which time she had an acute severe illness with high fever and marked loss of weight. For two weeks she was apparently "asleep," as the family described it. Her head was drawn back so that the occiput touched the back and it was impossible to insert the hand between the back of the head and the spine. The patient was ill for

*This case was published as a Medical Clinic by Dr. Thomas McCrae and Dr. Edward Weiss in the Philadelphia number, Medical Clinics of North America, January, 1931.

about three months. The mother states that there was no rigidity of the arms or legs. Following the illness it was noted that the patient gained weight very rapidly.

The mother was very emphatic in the belief that so far as they knew the child was normal until the time of this illness. As she recovered from the acute features the mother noticed that there was a change in both eyes, squint, which she thought gradually became more marked. There was also complete loss of speech for some time but exactly how long was not known. They stated that there had been considerable improvement in her speech recently; she was apparently learning more words and was able to articulate more clearly.

Her vision had become progressively worse since this illness and at the time of observation she could see only large objects. She had never gone to school because of her deficient vision. Her weight had steadily increased since the age of four. Her first and only menstrual period (up to the time of admission to the hospital) occurred in June, 1929, and was said to have been fairly profuse.

After the illness the patient was difficult to manage for a period of about six years and required constant watching. One striking feature was a voracious appetite, so that she consumed everything edible which she could find. She showed no special preference for sweet foods. Figure I is a photograph of the child taken about three months after the acute illness. She was plump but not unduly fat. There was strabismus of the left eye.

There was no history of headache or of any disturbance in the respiratory or digestive systems. It was stated that she usually drank very large amounts of water and urinated frequently. As a rule she voided twice in each night. It was not known how long she had had this polyuria. There was no history of enuresis or of incontinence of urine. We did not find that she took large amounts of water or had polyuria.

Present Illness. There was no account of any special recent symptoms different from those in the past history. For the past five years she had been much as she is at present but less difficult to manage than from the age of four to eleven.

Examination. On admission to the hospital the patient weighed 194 pounds and measured 151 cm. in height. The span was 143 cm. and the measurement from crown to symphysis pubis was 79 cm.

The patient was very stout and the fat was fairly equally distributed. There were no special pads above the clavicle or about the ankles. The hair seemed natural. The skin was perhaps a little dry. There were four fingers and a thumb on each hand and the fingers showed no special alteration from the normal but there were six toes on each foot. The second toe was longer than the great toe and all were spatulate. The finger nails were small and the toe nails exceedingly small. The eyebrows were not very well developed and were very thin on the outer third. There was a double convergent squint which was not constant in degree. The palpebral fissures were small. On throwing a light on the eyes there was a marked lateral nystagmus which was rapid and of considerable extent. The tongue was clean, the palate high and narrow. The teeth showed no abnormalities. The neck was fat and the thyroid gland could not be felt. There was asymmetry of the breasts, the right being larger. The axillary hair seemed normal. There was a moderate growth of hair on the arms and legs; there was no hair on the face. Examination of the heart, lungs, and abdomen gave no abnormal findings. The reflexes were practically normal.

She answered simple questions fairly well and was perhaps most intelligent in answering inquiries about her meals. Her voice had a curious sing-song quality with a high pitch and she often spoke in an explosive manner. It was noticed that she did not follow with her eyes an object held before her.

The report of the eye examination by Dr. Olsho follows: "There is marked alternating convergent squint and partial paralysis of the external rectus on each side. The media are clear, the disks paler than normal and the arteries small. Many areas of retinal pigmentation are found in each fundus. She can count fingers at 6 inches. Sometimes she seems to use only the nasal side of each retina. Pupils O.D. 3½ mm., O.S. 4 mm., reactions normal. The palpebral fissures are narrowed, due to obesity. It is not possible to determine the visual fields."

The diagnosis was partial paralysis of the external rectus of each eye and retinitis pigmentosa.

A pelvic examination was made by Dr. Brooke M. Anspach, who reported: "There is an abundance of pubic hair normally distributed. The mons veneris and upper greater labia are normal; the lower greater labia are not so well developed. The clitoris and prepuce are normal. The lower smaller labia are

poorly developed. The urethral orifice is farther away from the clitoris than usual. The vestibule is long. The hymen is intact; the orifice barely admits the little finger. Bimanual rectal palpation shows the uterus below the average size and in retroversion, lying in nearly the same axis as the vagina. The adnexa cannot be felt distinctly; probably they are small and ill-developed. The patient is very fat and this would make the palpation of normal adnexa difficult. The cervix is poorly developed. It is conical rather than rounded and in the center is a small bilateral split with some eversion of the cervical mucosa. The vaginal fornices are shallow. Diagnosis: Hypoplasia of the genitalia of moderate degree."

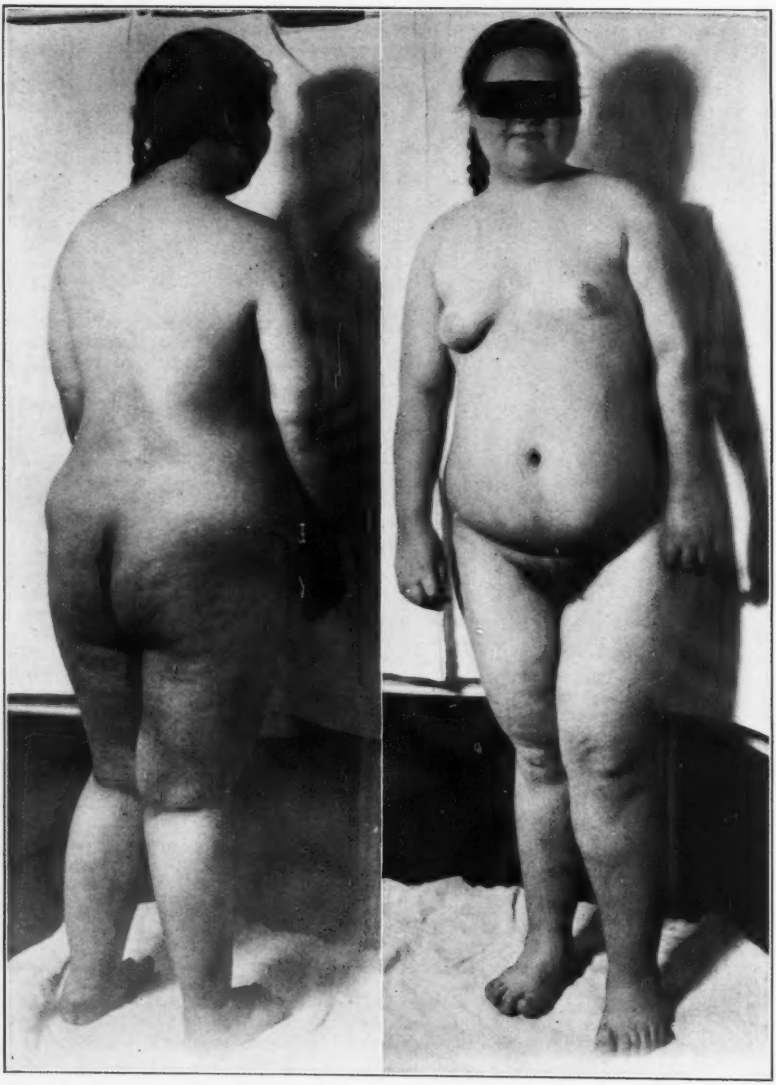


Fig. 2. Age 15. Note adiposity and extra toe.

*Psychometric Tests:** "The patient's life age is fifteen years and eight months but her mental age is approximately five years. Her intelligence quotient is approximately 35. She classifies as low grade feebleminded. She is very inferior in all mental functioning and below the level required for success in the first grade of school."

The various studies gave the following results:

The temperature was normal. The pulse rate varied from 80 to 100. The respiration rate was normal. The systolic blood pressure varied from 110 to 125 and the diastolic from 70 to 80.

We did not observe any evidence of excessive thirst or polyuria. The amount of urine usually averaged about 1200 cc. in twenty-four hours and the largest amount was 1600 cc. The specific gravity varied from 1.008 to 1.022. Traces of albumin were found on some examinations but no casts. Sugar was never found.

The blood count was normal. The blood sugar was between 0.10 and 0.116 per cent. The blood urea was normal in amount. The Wassermann and Kahn tests were negative. The spinal fluid was normal in every way. The serological tests were negative. The basal metabolism gave figures of minus 5 and minus 12. The sugar tolerance test gave results within normal limits.

Roentgenological examination showed the sella turcica to be perfectly normal. There were no anomalies in the bones of the skull. The first phalanx of the extra toes articulated with a separate head of the fifth metatarsal bone. The epiphyseal developments seemed to be normal for her age and ossification seemed to have proceeded normally in the hands and wrists.

DISCUSSION

From the study of our cases and a review of the literature it seems clear that we are dealing with a congenital and familial form of cerebral adiposity associated with mental deficiency, genital dystrophy, retinitis pigmentosa and frequently polydactylism. The recorded cases resemble one another very closely, a distinctive feature being the familial occurrence. In fact, Boenheim (7) states that cases can be placed in the Laurence-Biedl group only if familial. He also calls attention to the fact that one or another of the chief symptoms may be absent and that not all cases show the complete picture of the Laurence-Biedl syndrome as above described. In addition he mentions hardness of hearing as a cardinal characteristic of the syndrome.

Most of the discussion in the more important contributions to the subject has had to do with etiology and pathogenesis. The early students of the subject considered a lesion of the pituitary gland responsible; Biedl and Raab (8) discussed the development of the disorder from a mechanical standpoint, suggesting that a high and massive dorsum sella (a skeletal defect which they consider similar to that of polydactylism) interferes with the flow of an energizing secretion from the pars intermedia of the pituitary gland through the stalk of the infundibulum to certain metabolic and genito-tropic centers at the floor of the mid-brain.

Solis-Cohen and Weiss (1) looked upon Biedl's hypothesis, discussed in detail by Raab, as attractive but preferred to await further developments before arriving at any conclusion.

Just as the main objection to the pituitary gland origin of this syndrome was the absence in many cases of any evidence that could be interpreted as pointing to a lesion of this gland, so the main objection holds to

*I am indebted to Esther Katz Rosen, Ph.D., for the psychometric tests on this patient.

the Biedl and Raab hypothesis; no evidence of a high or massive dorsum sella has been found in many cases of the fully developed syndrome.

Ornsteen (6) in a recent paper offers a more satisfactory explanation based upon the embryologic development of the hypothalamic region of the diencephalon. He holds that a genetic defect of the prosencephalon (forebrain) is responsible and explains that the development of the infundibulum, the optic chiasm, and the retinal fibres is an intimate one. The ventral segment of the ectopic zone (a narrow area of the forebrain surrounding the optic vessicle) gives origin to all of them. From the dorsal area of this zone develops the epithalamus, thalamus, subthalamus, and geniculate bodies of the diencephalon. From its ventral area the hypothalamus takes origin. The cephalic area of the ectopic zone gives rise to the end brain. Hence a defect in the character of the genes responsible for various steps in the evolution of the ectopic zone in its ventral segment may result in an abiotrophic condition of the infundibulum, optic nerves and retinae. The infundibular defect results in the Fröhlich picture, the chiasmatic defect produces the optic atrophy and the pigmentary degeneration of the retinae is explained through failure of development of certain fibres in the optic chiasm. Since the cephalic segment of the ectopic zone gives origin to the end brain, it may be that the defect in intellectual development is a part of this segmental embryonic deficiency. The disorder, therefore, is to be considered on the basis of a genetic defect involving a developmental center of wide influence. The adiposo-genital dystrophy, retinitis pigmentosa and mental deficiency are related to a genetic defect of the ectopic zone in its cephalic and ventral segments; in other words, a cerebral genotypic unit character. The polydactylism, skull deformities, atresia ani and other occasional anomalies are present only through the accidental coupling of somatic genotypic unit characters.

Concerning the question of treatment of this disorder a few observers report beneficial results from organotherapy. Bernhardt (9) reports the case of brothers with the fully developed syndrome, aged 16 and 12 years, respectively, who were greatly benefited by the administration of thyroid, anterior pituitary lobe and calcium. The weight was reduced, basal metabolism was increased, and the muscles developed in both boys. In the older boy there was improvement of vision and genital development. They both became more active and attentive.

In one of Biedl's (3) cases treated with thyroid and pituitary extract, the vision improved, menses were reestablished and the patient lost 13 pounds in weight. The brother of the patient also improved on the same treatment.

De Schweinitz (10) also reports beneficial results in two of his three cases. He states that one of them responded well to organotherapy; vision, general condition and mentality showing distinct improvement. In the other case vision, which was rapidly declining, was maintained by pushing glandular feeding.

In Beck's (11) cases, a brother and sister, aged 11 and 10, respectively, the results of organotherapy, which consisted of thyroid and anterior pituitary lobe, were stated as very satisfactory, especially with respect to improvement in the mental condition. Treatment was given over a period of five months. General improvement continued and the improvement in mentality was checked by psychometric tests. Beck concludes that if the disease is recognized and proper treatment instituted much can be done to arrest its progress and in some cases improvement may occur.

The original subjects reported by Solis-Cohen and Weiss were not benefited by organotherapy, although it must be said that the patients did not satisfactorily adhere to treatment.

The present patient apparently has been benefited; a total weight reduction of 30 pounds occurred from the time of admission to the hospital (October 9, 1929) to June, 1930, and there was a surprising improvement in the mental state. Treatment was begun with 2 grains of whole pituitary gland extract and 1 grain of thyroid gland extract. After two weeks the thyroid gland alone was given in doses gradually increasing from 1 grain to 5 grains a day. During the 11 weeks of residence in the hospital the patient was on a limited diet of about 1500 calories and lost weight at the rate of a little over 2 pounds a week.

SUMMARY

The Laurence-Biedl syndrome is a congenital and familial form of cerebral adiposity associated with mental deficiency, genital dystrophy, retinitis pigmentosa and frequently polydactylism. A case is described, making a total of 48 cases so far recorded.

Early students considered that the condition resulted from a disorder of the pituitary gland; recently the tendency has been to regard a lesion of certain metabolic and genito-tropic centers in the floor of the midbrain as responsible for the development of the syndrome. This is explained embryologically as dependent upon a genetic defect of the forebrain. Treatment with glandular extracts, chiefly thyroid and pituitary, apparently has brought about improvement in some cases. This was true of the case here recorded.

Addendum: Since the preparation of the above article two more cases have been recorded and are added to the bibliography as references 12 and 13. These are included in the total of 48 cases mentioned in the summary.

BIBLIOGRAPHY

1. Solis-Cohen, S. and E. Weiss: Am. J. M. Sc. **169**: 489. 1925.
2. Laurence, J. Z. and R. C. Moon: Brit. Ophth. Rev. **2**: 32. 1866.
3. Biedl, A.: Deutsche med. Wchnschr. **48**: 1630. 1922.
4. Ricaldoni, A. and A. Isola: Arch. de Med. des Enfants, **32**: 27. 1929.
5. Bardet, G.: These de Paris, **107**: 470. 1920.
6. Ornsteen, A. M.: Am. J. M. Sc. To be published.
7. Boenheim, F.: Endokrinologie, **4**: 263. 1928.
8. Raab, W.: Wien. Arch. f. inn. Med. **7**: 443. 1924.
9. Bernhardt, H.: Ztschr. f. klein. Med. **107**: 488. 1928.
10. De Scheweinitz, G. E.: Tr. Am. Ophth. Soc. **43**: 12. 1923.
11. Beck, H. G.: This Journal, **13**: 375. 1929.
12. McKinney, J. McD.: J. Nerv. and Ment. Dis. **74**: 50. 1931.
13. Griffiths, G. M.: J. Neurol. and Psychopath. **12**: 53. 1931.

SOME CLINICAL ASPECTS OF A DIMINISHED CALCIUM UTILIZATION*

WALTER TIMME, M.D.
NEW YORK

The question of the importance of proper calcium ultilization and the effect of a disturbance therein have become of exceeding interest in the past two decades. From the time of the experiments of McCallum and Voegtlein in 1909 in which was demonstrated the relationship existing between calcium mobilization, parathyroidectomy and convulsive seizures, our knowledge has grown rapidly, and many hitherto obscure physical signs and symptoms—objective as well as subjective—have now been classified under this group of disturbances. It is interesting to note how gradually and at times purely accidentally the grouping came about. Thus, we had at the Neurological Institute in New York City for a number of years a certain type of case of adolescents that was repeated time and time again with the following set of seemingly unrelated complaints: (1) fatigue; (2) slowness of growth; (3) conduct disorders. One or all of these symptoms were the reason for appearing at the clinic. Year by year we have been adding to the group and endeavoring to classify its outstanding features. Upon examination, there appeared many correspondences in their physical signs and laboratory examinations; their musculature was the seat of extreme myotatic irritability and myoidema; their blood pressure was above normal level for age; their reflexes were uniformly exaggerated; muscle contractions were obtained at low levels of current. Laboratory examination disclosed in about ninety per cent of the cases a blood calcium below 7.0 milligrams per 100 cc. of whole blood. An interesting correlative fact was that the blood sugar was moderately high—in some cases up to 150 mgm. X-ray examination also showed interesting findings. Within the skull particularly, many cases showed deposits probably of calcium in the choroid plexuses, in the falx and in the pineal gland—although such deposits ordinarily ought not to occur before middle life. The deposits in the pineal perhaps accounted for some of the symptoms shown in the genital domain of various patients. Thus precocious puberty, i. e., enlarged genitalia were present out of proportion to the usual incidence of these conditions.

Since the beginning of our studies on these cases—some fifteen years ago—other occasional signs and symptoms manifested themselves—particularly in both the smooth and striped musculature domains. These had to do largely with an increased irritability, or oversensitiveness and lowered threshold to stimuli, and this led to spasticities. Hence spastic colitis was

*Read before the Fifteenth Annual Meeting of the Association for the Study of Internal Secretions, Philadelphia, June 8, 1931.

TABLE 1.

Observations on Fourteen Children Under Sixteen Years of Age.

Name	Age	Sex	Blood Calcium	Myotatic Irritability, Myoidema	X-Ray Examination	Other Glandular States	Behavior	Other Symptoms
G. M. W.	15	M	6.1	Excessive	Pineal calcified; small sella; thick skull tables	Enlarged genitalia	Manic outbursts; uncontrollable	Great fatigue
J. G.	11	M	6.2	Excessive	Pineal calcified; small sella		Assaultive; incorrigible; threw knife at father	Great fatigue; muscular cramps
R. Go.	11	F	7.1	Very marked	Pineal calcified	Enlarged clitoris and breasts	Incorrigible; assaultive; curses	Fatigue
R. Gn.	11	F	6.8	Very marked	Pineal calcified; very small enclosed sella		Quarrelsome; assaultive; unmanageable	Fatigue
V. S.	14	M	7.5	Marked	Pineal calcified; thick skull tables	Enlarged genitalia	Tenseness; easily angered; stammers	Fatigue; still has deciduous teeth
D. H. E.	16	M	7.8	Very marked	Small, enclosed sella		Assaultive; forges checks; police case	Fatigue
N. S. L.	16	M	7.1	Excessive	Pineal calcified	Thyroid enlarged	Incorrigible; easily angered; curses	Fatigue
M. F.	15	M	7.2	Very marked	Pineal calcified; small enclosed sella; thymus enlarged		Irritable; incorrigible; stammers	Fatigue
J. W.	9	M	6.2	Excessive	Small sella; convolutional digitations		Impatient; easily angered; quarrelsome	Fatigue; headache
J. M.	15	M	7.2	Excessive	Craniostenosis; oxycephalic; thick skull tables	Thyroid full	Easily excited and keyed-up; extreme tenseness	Fatigue; tenseness of muscles of neck
J. G.	12	F	6.8	Excessive	Pineal calcified	Menses at 11; pubertas praecox	Incorrigible; violent temper; curses	Thyroid full
A. B.	14	M	6.9	Very marked	Pineal calcified; thymus enlarged		Incorrigible; undisciplined; assaultive	Retarded; I. Q. 80%; fatigue
M. C.	14	M	5.9	Excessive	Pineal calcified; thick skull tables	Enlarged genitalia	Violent temper; irresponsible	Poor muscular coordination
V. A.	7	F	5.9	Marked	Digitations more than normally visible		Intelligence quotient 58%; cannot talk; excitable	Fatigue

TABLE 2.

Observations on Adults.

Name	Age	Sex	Blood Calcium	Myotatic Irritability, Myoidema	X-Ray Examination	Other Glandular States	Behavior	Other Symptoms
A. P. D.	29	M	5.4	Marked	Enlarged pituitary	Thyroid full	Easily aroused and then so blocked he cannot talk; excitable	Fatigue; trismus; muscle cramps
E. C.	26	F	6.0	Marked	Pineal shadow; small pituitary		Cannot concentrate; mind tense and immobile	Fatigue; scant menses; tense
C. B. D.	46	F	6.8	Marked	Exceedingly thick skull tables		Excitable; uncontrollable	Fatigue; urticaria; tense; no relaxation

TABLE 3.

Observations on Four Patients With an Apparently Normal Blood Calcium.

Name	*Age*	*Sex*	*Blood Calcium*	*Myotatic Irritability, Myoidema*	*X-Ray Examination*	*Other Glandular States*	*Behavior*	*Other Symptoms*
(*a*) J. L.	5	M	10.0	Marked	Persistent thymus needing x-ray at 8 months; sella quite small		No self-control; violent temper; tantrums	Fatigue
(*b*) J. Y.	9	M	10.6	Marked	Persistent thymus; x-ray treatment of thymus at 8 months		High temper; uncontrollable; violent; lack of self-control; domineering	Fatigue
(*c*) G. B.	8	M	10.5	Myoidema	Sella slightly smaller than usual		High temper; deficient; irritable	Fatigue
(*d*) J. C.	14	M	9.9	Marked	Pineal calcified; thymus present	Large genitals	Assaultive; bad; incorrigible; undisciplined	Fatigue

frequently met with; a constant irritating cough was an occasional finding for which no cause could be found until the calcium determination disclosed the probable reason in the spasmodic contractions of the pharyngeal group. Constant muscular tension without the ability to repose was a frequent complaint, and was possibly at the basis of the seemingly undue fatigue.

Recently Aub and Salter found that the spastic colitis due to lead intoxication could be exceedingly diminished or the pain thereof mitigated by parathormone injections. This result they attributed to the relief of the spasticity due to calcium metabolic disturbances induced by lead.

Uterine spasms with pain can to some extent also be credited to the calcium disturbance. The reason underlying this is perhaps the fact that menstrual blood, being rich in calcium—up to 16 or 18 mgm. per 100 cc.—causes much calcium loss. At any rate, therapy on this basis helps in many cases.

Because of the extreme fatigue or the general body tenseness, these patients lose weight, their musculature shows an attenuated character with very little sub-cutaneous adipose tissue so that the joints are unduly prominent. Added to this, we frequently encounter the spasmodic cough alluded to; x-ray examination of the thorax gives the evidence of calcium deposits in the bronchial lymph nodes and a clinical picture is finally evolved which much resembles a generalized tuberculosis.

Several instances of this type have since been recognized at one of our sanitaria for tuberculosis and the diagnosis of that disorder has been withdrawn.

But from our point of view, the most important groups of symptoms were those developed from the nervous system. Previous investigations had shown that both the central nervous system and the vegetative—with a blood calcium deficiency—gave evidence of increased sensitiveness to stimuli, the lowered threshold probably being at the synapses. At any rate, children, and adolescents particularly, thus affected, reacted with marked rapidity to all types of stimuli and at the same time more intensely than the average. These patients were inordinately affected by environmental changes, temperature variations, noises and disturbances of all kinds; opposition and criticism irritated them and they reacted rapidly to them. And so, because of some words of admonition or fault-finding or even a glance or attitude denoting this, such a patient would become violently agitated—out of all proportion to the cause. He would shout, or scream, or strike, or throw things about, and attack his aggressor without any sense of judgment arising to inhibit these rapid responses. One of our patients, in such a moment of disturbance, threw his sister out of a window because of some slightly disparaging remark she had made; another one shot at a group of his schoolmates with a gun that was handy at just the right—or, rather—wrong moment. Immediately after the act, contrition arises, but too late. Thus arise many criminal cases, assaultive and homicidal in

character. Another group is comprised of those who show quickness and alertness in responsive speech—in other words, are witty. These witty responses are almost never the result of thought processes but are due to rapid awakening of associations of many kinds, and the retort is actually made before the talker is aware of what he has said.

TABLE 4.

OBSERVATIONS ON PATIENTS UNDER SIXTEEN YEARS OF AGE.

BLOOD CALCIUM		BLOOD SUGAR	
5 to 6 mg. per 100 cc.	6	120 to 140	5
6 to 7 mg. per 100 cc.	9	110 to 120	7
7 to 8 mg. per 100 cc.	4	100 to 110	1
8 to 10 mg. per 100 cc.	4	Below 100	2
BLOOD PRESSURE		PINEAL SHADOW	
Above 120	5	Present	14
110 to 120	5		
100 to 110	4	Absent	5
Below 100	3		

GROWTH DEFECTS

4 inches (10.16 cm.) or more below average height for age	9
2 to 4 inches (5.08 to 10.16 cm.) below average height for age	4
Normal	6

These behavior types vary from the mildly irritative through the series of various grades of incorrigibility to assaultiveness and to even hypomanic outbursts with homicidal attempts.

In the domain of the vegetative nervous system likewise objective symptoms as well as subjective make their appearance. We have tried, and still have reached no definite conclusion, to determine whether there is clinically demonstrable a measurable disturbance in the true sympathetic or in the parasympathetic divisions of the system. We may say at this time tentatively that the calcium sub-utilization condition does not specifically affect either one to the exclusion of the other, but that it accentuates the individual predilection of the patient. That is to say, if a so-called vagotonic state is present *ab initio,* the lack of calcium makes a markedly vagotonic status of a moderately latent one; and similarly with the sympathetic type—the calcium disturbance accentuates all its symptoms. To particularize; while all the patients in the group became readily excited, the vagotonic ones went into a blue funk, became easily depressed and perspired freely on such occasions. Fainting was a frequent occurrence with them. Pilocarpine in small doses caused much nausea and gastrointestinal distress. Of the vagotonic group but very few individuals were seen.

The subjects of sympathetic types of irritation became aggressive, acquired a rapid pulse, and complained of anginoid chest pains, throbbing in the neck (thyroid?) and a feeling of fulness and heat in the head. By far the greater number of patients are grouped in this latter class. Adrenalin and atropin accentuated this condition.

The blood calcium findings in these cases were with few exceptions quite below the low normal values but not down to the tetany level. They were usually between 5 and 7 mgm. per 100 cc. of whole blood. The ex-

ceptions had a normal blood calcium value but because the symptomatology of these cases coincided with those that were definitely hypocalcemic they were treated on the same basis excepting that no calcium was included in their treatment. The fact of the presence in the blood of a certain amount of calcium was not tantamount, in our opinion, to its utilization in corresponding degree, and the problem of treatment in such cases therefore was one of increased utilization. Without going deeply into the details of treatment it may suffice to say that a few years ago the administration of calcium in various forms was at first all that was done. A few patients apparently reacted favorably to this. Later, when we had decided that the ultilization of this calcium was the important factor, we used general glandular products—thymus gland and parathyroid in addition—but with little added success, although the conduct vagaries seemed in a measure occasionally to clear up. A few years ago when the Collip parathormone preparation was added to our armamentarium we had for the first time a dependable instrument. Injections, varying in strength and time of administration suitable to our cases, caused almost an immediate change in the varying symptomatology which we had looked upon as dependent on a low calcium utilization. Especially when combined with large doses of calcium the effect of its administration was quickly apparent. The subjective sensation of a lack of muscular tension with attendant relaxation was stated to have occurred shortly after the first injection. As a consequence of this lessened tension, irritability decreased, fatigue disappeared and sleep became more normal. The conduct disturbances became much modified and in some cases after a week or two of treatment practically disappeared. To control these results, treatment with the injection was discontinued for weeks at a time and some other bland injection substituted. The irritability with its attendant disturbances returned within a fortnight of the discontinuance. We have not yet determined how soon after a complete series of treatments, recurrences of symptoms take place. Our custom has been to keep up the injections for a few months with intervals of a week's freedom from time to time; then gradually reducing the treatment weeks to two and finally to one a month, with, however, administration of calcium throughout the period. The summer months with plenty of sunshine have been selected for discontinuance of treatment entirely. Our final results will be published in the course of the next year or two. Thus far, the outcome of treatment has been beyond our expectations.

A word might be added in reference to our experience with other calcium mobilization agents, notably, with those containing vitamins B and D. To relieve the patients of the necessity for frequent hypodermatic injections of parathormone, we substituted in some these latter medicaments, with practically no success at all. Viosterol produced on the contrary increased irritability in many cases and greater tenseness. When combined with the parathormone injections the good effect of the latter was possibly enhanced, but without parathormone our therapeutic endeavors could be

classed, with but a few exceptions, at the level of the simple calcium administration of years ago. This observation more or less confirms that of Quick and Hunsberger* of some antagonistic action existing between the parathyroid and viosterol.

SUMMARY

A report is made of a rather well defined group of patients with a symptomatology strikingly similar in all cases. The basic disturbance seemed to be a lack of calcium utilization due to parathyroid deficiency which resulted in secondary disturbances in the glandular system and, particularly, in behavior disturbances of marked degree.

Treatment is of marked usefulness and many cases go to apparent cure.

*J. A. M. A. 96: 745. 1931.

Abstract Department

The mechanism of epinephrine action. V. Changes in liver glycogen and blood lactic acid after injection of epinephrine and insulin. Cori, Gerty T., C. F. Cori and K. W. Buchwald, J. Biol. Chem. **86**: 375. 1930.

Blood sugar, blood lactic acid, and liver glycogen were determined in well fed rats at various time intervals after subcutaneous injection of epinephrine, insulin, or both hormones. Fifteen minutes after the epinephrine injection liver glycogen was lower, after 1 hour it was the same, and after 3 hours it was higher than in control animals. Blood lactic acid which was augmented after 15 minutes and after 1 hour, had returned to normal 3 hours after the injection, while blood sugar was elevated at that time. On the basis of the available data it was calculated that the sugar set free in the liver in the first hour of epinephrine action amounted to only one-fourth of what a rat can use per hour. Fifteen minutes after the insulin injection liver glycogen and blood sugar were unchanged, while after 1 hour both had diminished. When insulin and epinephrine were injected, the former prevented a decrease in liver glycogen and the increase in blood sugar regularly observed after the injection of the latter. Insulin is therefore an inhibitor of the hepatic glycogenolysis accelerated by epinephrine. Insulin has, however, very little effect on the increase in blood lactic acid resulting from the injection of epinephrine.—Authors' Abst.

The effect of extract of adrenal cortex on the uterus (Ueber die Wirkung von Nebennierenrindenextrakt auf den Uterus). Engelhart, E., Klin. Wchnschr. **9**: 2114. 1930.

This author finds that lipoid extracts of adrenal cortex prepared by Corner's method of extraction of corpus luteum when injected subcutaneously into virgin rabbits cause a three-four-fold increase in size in the uterine musculature and mucosa. Whether the effect is due to cholesterin-like substances or is specific for adrenal cortex is subject to further study.—E. P. D.

Effect of adrenalin on gluconeogenesis. Mobilization of muscle glycogen by adrenalin (Einfluss des Adrenalins auf die Zuckerneubildung. Mobilizierung des Muskelglykogens durch Adrenalin). Geiger, E. and E. Schmidt, Arch. f. exper. Path. u. Pharmakol. **143**: 321. 1929.

In 5 phlorhizinized dogs adrenalin injection caused a rise in the blood lactic acid level from an average resting value of 25 mgm. % to 92, 95, 100, 59 and 97 mgm. %. When the same dogs were again injected with adrenalin after 2 days, the rise was not as conspicuous or even failed to occur. The following values were observed: 50, 35, 35, 34 and 25 mgm. %. The conclusion is reached that the blood lactic acid has its origin in muscle glycogen and that the first injection of adrenalin depletes the glycogen stores so that the second injection is less effective. Blood lactic acid was determined by means of the method of Dische and Laszlo.—G. T. Cori.

Tumors and adrenalectomy (Tumores en ratas suprarrenoprivas). Laclau, N. C., Rev. Soc. argent. de biol. **6**: 573. 1930.

Bilateral adrenalectomy in white rats does not influence growth of tumors, but the animals are more sensitive to cachexia.—J. T. Lewis.

On the antagonistic action of adrenalin and insulin on blood sugar regulation. Experiments on adrenalectomized dogs (Ein Beitrag zur Frage des Antagonismus von Adrenalin und Insulin bei der Blutzuckerregulation. Versuche an nebenniernlosen Hunden). Meythaler, F. and R. Cario, Arch. f. exper. Path. u. Pharmakol. **154**: 193. 1930.

In dogs in morphin-ether anesthesia all vessels leading to and from the adrenals were ligated. Injections of 5 cc. of a 10 to 60% glucose solution injected into pancreatic-duodenal artery led to a transient rise and then to a drop of the blood sugar level. The same solutions injected into the femoral artery caused a rise of the blood sugar. Ardenalin injected into the pancreatic artery raised the blood sugar. These results are taken to prove that glucose is the "hormone" of insulin secretion.—G. T. Cori.

Studies of the functions of the adrenal cortex (Studien über die Funktion der Nebennierenrinde). Reiss, M., Endokrinologie **6**: 321. 1930.

The author shows that the life of an adrenalectomized animal can be lengthened by administration of a substance free from the blood pressure factor of the adrenal. The substance is successful only if administered before the typical adrenalectomy symptoms appear. This condition can not be directly due to the hormone condition of the cortex since injection of greater masses of the extract are ineffective. The reactions once initiated by adrenalectomy are irreversible; the extract only hinders the process if administered in time.—B. C.

The function of the adrenal cortex (Studien über die Funktion der Nebennierenrinde). Reiss, M., Endokrinologie **6**: 421. 1930.

The author confirms previous work which showed death followed adrenalectomy in decerebrated cats and dogs. Studies made on respiration showed death to be due to respiratory failure. However, when artificial respiration was resorted to, death ensued after about the same interval. Injections of the cortex hormone prolonged life. The reserve alkali decreased after adrenalectomy, regardless of whether or not artificial respiration was used; the reserve was increased upon the hormone injection. The reduction of the alkali reserve seems to be due to a disturbance of metabolic materials for which the active factor has not as yet been found.—B. C.

Adrenalin in the treatment of contraction ring, with note on the action of adrenalin upon tubal contractions. Rucker, M. P., South M. J. **24**: 258. 1931.

Kymographic tracings made with the Rubin apparatus show that injection of adrenin hypodermically causes a relaxation of the musculature of the tube with often a slowing of the contraction rate. The writer summarizes as follows: The history of the discovery of the action of adrenalin upon the human pregnant uterus is traced as well as the development of the practical application of this knowledge. For the extreme grades of contraction rings adrenalin is the only remedy that permits of a delivery through the birth canal. Moderate grades of contraction rings occur not infrequently and are the cause of dystocia, often where it is the least expected. Adrenalin in these cases will make forceps extraction easy. The use of adrenalin in threatend abortions is logical and is worthy of further trial.—J. C. D.

The influence of the endocrine glands upon the sulphur metabolism in the urine of dogs. IV. The influence of adrenalin (Ueber den Einfluss der endokrinen Drüsen auf den Schwefelstoffwechsel in Harn des Hundes. IV. Mitteilung. Ueber den Einfluss des Adrenalins). Tasaka, S., Folia Endocrinol. Japon. **6**: 107. 1931.

The author has investigated the influence of a subcutaneous injection of adrenalin upon the sulphur partition in the urine. For this purpose 3 healthy, grown bitches, which were fed upon a special ration, were used. After the subcutaneous injection of adrenalin the nitrogen and sulphur metabolism were greatly stimulated. The sulphur metabolism was more strongly influenced than the nitrogen and the increased output was greatest on the day following the injection. The neutral sulphur was increased the most, the ethereal sulphuric acid next, and the inorganic sulphuric acid the least. On the second day after the injection of adrenalin this increased nitrogen and sulphur excretion had nearly returned to normal, but the percentage ratio of the neutral sulphur and ethereal sulphuric acid to the total sulphur still remained somewhat higher. On the third day after the injection these ratios were all normal.—J. Gagnon.

Choline and the adrenal gland (Choline e ghiandola surrenale). Viale, G. and T. Combes, Arch. di Fisiol. **28**: 25. 1930.

From experiments on dogs the author concludes that the injection of adrenalin (1 cc. of 1:20,000) causes an outpouring of choline from the adrenals into the circulation; the effect lasting less than ten minutes after the injection. Administration of the same quantity of adrenalin to adrenalectomized dogs did not produce this effect. He found that cutting the vagus unilaterally had no effect upon the choline content of the adrenal.—Katherine Brownell.

On the minute volume of the stomach: The influence of blood pressure, vagus stimulation, and organ extracts (Über das Minutenolumen des Magens und seine Beeinflussung durch Blutdruck, durch Vagustrizung, durch Histamin und Organ-extrakte). Bolhnheim, F., Ztschr. f. d. ges. exp. Med. **71**: 88. 1930.

The minute volume blood flow was found to average 26.4 cc. per 100 gram of stomach weight, with an average blood pressure of 60 mm. Hg. With an increase in stomach weight there is a relative decrease in minute volume. With an increase in blood pressure the minute volume flow increases, but the increase is not proportioned. Stimulation of the peripheral vagus in the neck decreases the volume flow; but this is not parallel with the fall in blood pressure, since the slowing of the venous flow occurs after the blood pressure has returned to its old level. Stimulation of the gastric vagus causes a more or less marked reduction in minute volume which is not parallel with the strength of stomach contractions produced by organ stimulation. The slowing of the venous flow is a direct effect and not due to an effect on the circulation. Hypophysin, euhormonal, insulin, histamin (subcutaneously) and thyroxin decrease the minute volume flow.—A. C. Ivy.

The physiological oxygen consumption of the stomach: The influence of vagus stimulation, histamine and organ extracts (Der physiologische Saverstoffverbrauch des Magens, seine Beeinflussung durch Vagusreizung, durch Histamin und durch Organextrakte). Bolhnheim, F., Ztschr. f. d. ges. exp. Med. **71**: 108. 1930.

The amount of work of the resting stomach of dogs and the effects of peristalsis and secretion were determined. The method consisted of comparing the oxygen content of the venous blood (collected from the gastric vein) with that of the arterial blood (femoral artery). The difference multiplied by the amount of venous blood which flows through the stomach (minute volume) was the measure of the work done by the stomach. The value for the oxygen used by the inactive stomach was found to be 169 cc. for 100 gram of stomach weight. With increase in stomach weight there is an increase in oxygen consumption. When peristalsis is induced by stimulation of the vagus there is a decrease in the oxygen content of the venous blood. Since the minute volume is decreased, there is actually a decrease in the amount of oxygen that is utilized when calculated on the basis of minute volume. Hypophysis extract and insulin cause an increase in oxygen consumption. Euhormonal and thymus are without effect. Histamine (subcutaneously) which stimulates gastric secretion, causes a decrease in minute volume and a marked increase in the amount of oxygen utilized. Thyroxin produced a decrease in minute volume and in utilization of oxygen. Suprarenin which decreases motility and secretion also decreases oxygen consumption.—A. C. Ivy.

Endocrine and other anomalies in anencephaly. Ettinger, G. H. and J. Miller, J. Obst. & Gynaec. Brit. Emp. **36**: 552. 1929.

Nine cases of anencephaly, of which 6 were females, were studied. The most important endocrine anomalies found involved the pituitary and the adrenals. The subcutaneous fat was in excess in all cases. No constant alterations were found in the thyroid, pancreas, spleen or ovaries. The pineal gland was never found. The parathyroids were not investigated. Using either Kiyono's or Browne's index for normal weight of the thymus no definite enlargement could be demonstrated. The cortex of the adrenal was found to be reduced when compared with normal fetuses of the same age. In 2 cases no traces of adrenals could be found. The pituitary was found in 8 cases. In only 3 of these was any pars nervosa demonstrated and that in very small amount. Other anomalies involved the viscera, particularly the kidneys, and the skeleton.—W. J. A.

Recent advances in Endocrinology. Prescriber **25**: 172-212. 1931.

An excellent review of some of the more outstanding recent work.
—R. G. H.

Hyperemesis and the endocrine glands (Hyperemesis und endokrine Drüsen). Frankl, O., Endokrinologie **7**: 167. 1930.

The author discusses possible endocrine factors in the pernicious vomiting of pregnancy, citing two fatal cases with a complete necropsy report in one and the more significant findings in the other. The first showed pathological changes in the corpus luteum, adrenal cortex, and liver, the latter showed fatty degeneration but no necrosis, a condition duplicated in the kidneys. In the second case, in which the immediate cause of death was an acute infection following abortion, the thyroid was adenomatous, the pituitary and parathyroids enlarged, and the liver showed fatty degeneration and infiltration together with necrosis. The author analyzes the potential endocrine factors and concludes that the true solution of the underlying etiology of hyperemesis gravidarum will only be gradually made clear as extensive investigation adds to our factual knowledge. In such studies, the corpus luteum must not be overlooked.—A. W. R.

Experiments to obtain hormones by use of a special method of Vivi-diffusion (Der Einfluss der Hyperinterrenalisierung auf die Entwecklung der weiblichen). Müller, C., Endokrinologie **7**: 401. 1930.

The experiments consisted of perfusion of the peritoneal cavity of the rabbit (morphine anesthesia) with physiological salt and tyrode solutions and the testing of the perfusate thus obtained for the presence of adrenalin and pituitrin by biological methods. The perfusate was tested on the living, isolated guinea pig uterus for pituitrin, on the living, isolated intestine of the rabbit for adrenalin. The animal was fastened to a board at an angle of 60°. A trochar was introduced into the upper part of the abdominal cavity through which the inflow, another into the lower abdominal cavity through which the outflow, were effected. The same animal could be used for these experiments for months. Neither adrenalin nor pituitrin could be shown to be present in the perfusate thus obtained. However, after injection of pituitrin (0.85 cc.) intramuscularly and adrenalin (2-4 cc. 1:1000) intravenously, the perfusate obtained contained these substances in appreciable amounts. The standards of comparison for pituitrin which was used was histamin, that for adrenalin a standard preparation of adrenalin. In a typical experiment 0.2 cc. perfusate contained 0.0017 mgm. adrenalin. These experiments show that normally pituitrin and adrenalin, if present in the perfusate, are not in sufficient concentration to be detected by the known methods. They further show that these 2 hormones are not changed nor destroyed as rapidly in the body as is commonly thought, since they may be obtained after artificial administration in the perfusate for as long as three hours' duration.—A. C. Ivy.

Observation of three cases of blue scleroticism with endocrine involvements (Sur trois observations de sclerotiques bleues ec troubles endocriniens). Nilus, F., Rev. franç. d'endocrinol. **9**: 3. 1931.

Two cases of a permanent type of blue scleroticism, and 1 intermittent case with sympathetic derangement were observed. Bone fragility was present in 1 case. In the other 2 cases a marked ligamentary laxity was found. There were no auditory troubles. In 2 cases the affliction was hereditary, the third was an isolated case. Hereditary syphilis was present in 1 patient but was absent in the other 2 patients. One of the subjects was afflicted with thyroid disorder, the other with thyroid and parathyroid derangements. The 3 cases observed confirm the importance of the study of the sympathetic-endocrine complex in "blue scleroticism."—J. Gagnon.

Endocrine factors in human sterility (Endokrine Factoren in der menschlichen Sterilität). Rowe, A. W., Endokrinologie, **7**: 81. 1930.

A preliminary statement of an investigation now under way dealing with the causes and correction of infertile matings. The approach is four-sided and involves the complete study of both partners by clinical and laboratory methods supplemented by equally thorough investigations of the wife by gynaecological, and the husband by urological procedures. The relative nature of infertility in man, the dual responsibility of the couple for the infertility, and the simultaneous presence of several possibly causal factors in each mating are among the

interesting general points elicited. It is shown that over 50 per cent of the series of 60 couples gave definite evidence of an endocrinopathy, either hypo- or dys-functional in character. Pituitary disorders were demonstrated in 20 males and 17 females, thyroid failure in 8 males and 4 females, and ovarian hypofunction in 9 females. The investigations of the author and his associates, based on the study of adult male castrates, have failed to demonstrate any significant endocrine activity of the testicle in adult years. As one factor in the causation of human infertility, endocrine disorders play an important rôle. The body of the article deals with the objective observations of the investigation.
—Author's Abst.

The basis and regulation of human constitution (Die Grundlagen und die Ordnung der menschlichen Konstitutionen). Saller, K., Endokrinologie, **6**: 241. 1930.

The author concludes that a constitutional type as related to species, race, family, sex or age, is based upon a natural scientific foundation, but that the idea of a "constitution type" as related to the norm is unscientific and based only on conventional agreement. He believes that hormones may affect the individual but not the species.—B. C.

Obesity, constitutional or endocrine? Silver, S. and J. Bauer, Am. J. M. Sc. **181**: 769. 1931.

The authors emphasize the constitutional nature of the obese individual and the congenital and hereditary nature of obesity. Attention is called to Bauer's previous studies in which only 2.6 per cent of 275 cases of obesity were due to endocrine dysfunction and that 88 per cent had a familial tendency toward obesity. The balance theory of obesity is also criticized.—E. L.

The relation of the endocrine organs to rickets and tetany. Stoltenberg, L., Norsk. mag. f. laegevidensk, **90**: 729. 1929.

The author produced rickets and tetany in rabbits by giving them a rachitic diet and by excluding sunlight from their cages. He then studied the pathologic anatomy of the thymus, suprarenal, thyroid and parathyroid glands. The thymus and suprarenal glands showed no changes that might be related to rickets or tetany. The thyroid gland showed the same picture as that caused by hypofunction of the gland: enlargement of the whole gland, and microscopically, a large number of epithelial cells and a small amount of colloid in the alveoli. There was no sign of degeneration. The parathyroid glands were always enlarged when the animals had been given the diet for several weeks. This enlargement was also considered as hyperplasia on account of the morphology of the cells. The author considers this hyperplasia of both thyroid and parathyroid glands as a hypofunction of both glands and states that this is one of the ways that the animal reacts to the insufficient diet.—M. B. G.

Endocrine disturbances in childhood. Talbot, F. B., South. M. J. **24**: 13. 1931.

This is a summary of our present information presented briefly gland by gland and accompanied by a chart showing the symptoms associated with the different disorders.—J. C. D.

The human ovary at laparotomy. Allen, E., L. J. Bland, J. P. Pratt and Q. U. Newell, South. M. J. **24**: 23. 1931.

The endocrine observations are summarized in the statement that considerable amounts of follicular hormone ("theelin," "estrin," "folliculin") can be demonstrated by injections and implants into test animals of fresh tissue from both follicles and recent corpora lutea. Corpora lutea of the first few months of pregnancy contain considerable amounts. Corpora of full term pregnancy contain little or none of this hormone which is, however, abundant in the placenta.—J. C. D.

The response to colloidal dyes by the cells of the guinea pig testis (Les cellules a fonction colloïdopexique du testicule du Cobaye). Bratianu, S., Compt. rend. Soc. de biol. **103:** 710. 1930.

Guinea pigs were injected with trypan blue and other dyes. These were engulfed by the histiocytic tissue of the intratubular areas of the testis. The true interstitial cells of Leydig did not take up the stain. In this failure to stain with certain colloidal dyes, these cells resemble those of other endocrine organs and can, therefore, be classed as secreting, not reticulo-endothelial cells.
—J. C. D.

Comparison of the effects of the urine of pregnant women on the male and female genital tract (Comparaison de l'action de l'urine de femme enceinte sur le tractus génital male et sur le tractus génital femelle). Brouha, L. and H. Simonnet, Compt. rend. Soc. de biol. **103:** 661. 1930.

The authors summarize their observations, presumably on mice. A single dose is effective in producing precocious development in the male, while divided doses are necessary in the female. The tracts of the 2 sexes have similar latent periods from the initial dose. Both show thresholds, which are similar, and a maximum growth in size, which is reached more rapidly in the male. Urine taken as early in pregnancy as the week following the first menstrual period missed produces this reaction.—J. C. D.

Investigations concerning the crystalline female sex hormones (Untersuchungen über das krystallisierte weibliche Sexualhormone). Butenandt, A., Klin. Wchnschr. **9:** 1475. 1930.

An active crystalline substance was prepared from the urine of pregnant women. The author believed it to be the oestrus-producing hormone. The substance melted at 245° C., and from analyses the formula $C_{23}H_{28}O_3$ or $C_{24}H_{32}O_3$ was ascribed to it. This crystalline substance (progynon) was unsaturated and on account of its behavior with aqueous alkali, it was believed to be a hydroxy lactone. Bio-assay showed that it had a potency of 8 to 10 million mouse units per gram.—E. A. Doisy.

The late results of a deferential fistula in the guinea pig (Résultats éloignes d'une fistule déférentielle chez le Cobaye). Champy, C., Compt. rend. Soc. de biol. **103:** 751. 1930.

The ductus deferens was cut and the proximal end sewed to the adjacent skin. The testis on the opposite side was removed. At the end of 3½ years, the animal resembled a castrate in body form, genitalia, condition of the seminal vesicles, and distribution of fat. The essential point was that the remaining testis showed almost entire atrophy of the tubular portion and an overgrowth of apparently normal interstitial tissue to at least ten times its usual volume.—J. C. D.

The hormone of the corpus luteum (Das Hormon des Corpus Luteum). Clauberg, C., Zentralbl. f. Gynäk. **54:** 7. 1930.

Clauberg believes that the data obtained from animal experimentation and from observation of normal and pathological uteri and ovaries of the human female indicate that the proliferation phase (terminology of Schroeder) of uterine cycle is produced by the follicular hormone; the secretion or premenstrual phase by the corpus luteum hormone. Further confirmation of this idea is presented by the author in the form of data obtained from administration of follicular hormone ("Menformon") and corpus luteum extracts to a series of castrate female rabbits (number not given). It was found that 240 mouse units of "menformon" (40 M.U. for 6 days) produced a condition in the uterus of the castrate rabbit comparable to that which is found 10-12 hours after copulation. The untreated castrate uterus shows typical castrate atrophy with decrease in amount of endometrium and muscle. The injection of a corpus luteum extract prepared according to the method of Corner and Allen in amounts equal to 0.12 cc. for 5 days results in a proliferation and dilatation of the glands and hyperaemia of the endometrium, a condition typical of the pseudogravid or gravid uterus. These findings are evidence for the author's conclusion that the follicular hormone is responsible for the proliferation and growth of the uterus in

contrast to the corpus luteum hormone which does not promote growth or proliferation but is a specific hormone of the pseudogravid or gravid phase of the rabbit uterus and the secretion or premenstrual phase of the human uterus.
—R. K. Meyer.

The preparation and assay of crystalline female sex hormone. D'Amour, F. E. and R. G. Gustavson, J. Pharmacol. & Exper. Therap. **40**: 485. 1930.

A method for the preparation of crystalline estrin is described. This crystalline material was assayed by injection of varying amounts into 100 ovariectomized rats and the response plotted. If the Coward and Burn rat unit is used, i.e., that amount necessary to bring 50 per cent of a large number into estrus, the rat unit of crystalline estrin was found to be .38 gamma.
—Authors' Abst.

Corpus luteum of pregnancy. Persistence of pregnancy after excision of the corpus luteum in the early weeks. Douglass, M., Surg. Gynec. & Obst. **52**: 52. 1931.

A case is reported wherein a corpus luteum of pregnancy was excised, the pregnancy being of approximately 5 weeks' duration. Previous curettage proved the duration of pregnancy. This pregnancy progressed to its second half and was followed by spontaneous rupture of the membranes and prolapse of the cord with spontaneous abortion of a normal foetus. Two incidental cases were added where the corpus luteum was excised, one of the 4th month followed by abortion, and the other in which pregnancy was not suspected. Case one is evidence to the effect that corpus luteum is not necessary to the extension of the pregnancy after the fifth week.—Author's Abst.

Autogenous grafts of uterine horn; their susceptibility to ovarian hormones (Autogreffes de trompe utérine; sensibilité aux hormones ovariennes). Guerriero, C., Compt. rend. Soc. de biol. **103**: 719. 1930.

In rabbits bits of uterine horn transplanted under the skin of the head showed over a period up to 7 months, changes in secretory activity and histology which were synchronized with similar changes in the undisturbed uterus.
—J. C. D.

A critical study of female sex hormone preparations. D'Amour, F. E. and R. G. Gustavson, J. Pharmacol. & Exper. Therap. **40**: 473. 1930.

This investigation was an attempt to explain discrepancies in results reported by different workers on the basis of varying techniques of assay. One hundred ovariectomized rats were injected with varying amounts of estrin, the results plotted and the rat unit determined. The variation in the rat unit when smaller groups were used was studied and the effect of weight and of priming determined. The conclusion of Coward and Burn that there may be a variation as great as 1000 per cent in the response to estrin injections was confirmed. This variation, in our opinion, renders assays invalid in which a small number of animals were used. The weight factor was found to be negligible. A smaller unit is obtained if priming is employed. The necessity of international agreement on a method for measuring estrin was emphasized.—Author's Abst.

Lactation and menformon (Lactation et menformon). de Jongh, S. E., Arch. néerl. de physiol. **15**: 471. 1930. Abst., Physiol. Absts. **16**: 131.

Male and female guinea-pigs were given 100 or more units of menformon daily subcutaneously for several weeks; on stopping the injections a flow of milk was observed lasting a few days. More prolonged secretion was obtained when the amount injected was reduced, but not completely stopped, followed by a gradual reduction of the dosage. It is considered that this latter procedure imitates more closely the falling content of menformon in the body of the human female after parturition.

Menformon and milk secretion (Milchsekretion und Menformon). de Jongh, S. E. and E. Laqueur, Klin. Wchnschr. **9**: 2344. 1930. Abst., Physiol. Absts. **16**: 131.

By suitable dosage of menformon milk secretion can be evoked in adult female or normal male guinea-pigs and may be made to continue for almost six weeks. Large doses of menformon stimulate growth of the mammary gland. If the dose is now suddenly diminished milk secretion ensues after 2 days, and continues if the dose is gradually diminished to simulate what probably happens normally after parturition.

Parabiosis, sex glands and sex gland antagonism (Parabiose, Geschlectsdrusen und geschlechtsspezifischer Antagonismus). Kallas, H., Endokrinologie, **6**: 188. 1930.

Injection of anterior pituitary into one of a pair of parabiotic rats caused a rapid growth in the female gonad of recipient, but not nearly so marked in the other. Ovarian tissue was transplanted into a normal male, which latter was placed in parabiosis with a castrated male or in some cases with a female. Using mammary glands as a check, decided changes became evident within 3 weeks in the castrated animal. This does not occur in the parabiotic male where recession of gonad and accessory glands occurs. However, in simple transplantation of an ovary into a normal male, examination of testicle, seminal vesicles and prostate show them to be enlarged rather than reduced. Microscopic examination showed spermatogenesis in full sway. The ovary transplant, which had been placed in the kidney, showed typical conditions. The author believes there is an antagonism between the male and female sex glands.—B. C.

The action of Prolan (Aschheim-Zondek) upon the male sex organs (Die Wirkung des Prolan [Aschheim-Zondek] auf die männlichen geschlechtsorgane. Auf Grund von Versuchen au Mane und Ratte). Kraus, E. J., Klin. Wchnschr. **9**: 1493. 1930.

The author injected immature male mice with "prolan" and noted a definite stimulation in the growth of the male accessory glands, prostate and seminal vesicles, and a marked stimulation of the interstitial cells of the testis. He was unable to detect any stimulating action of the injected substance upon the germinal tissue proper, since spermatozoa were not noted to appear at ages earlier than in uninjected controls, nor in greater numbers than found in the controls. Spermatogenesis therefore appears to be unaffected by the substance, hence the question is raised whether the substance really acts as a "motor" for the germinal tissue. He noted that introduction of folliculine along with prolan gave results attributable to prolan alone; no depressing effect from the female hormone upon seminal vesicles or prostate gland occurred when at the same time prolan was present in effective amounts.—C. R. Moore.

Does folliculin act on the ovary (La folliculine agit-elle sur l'ovaire)? Lipschutz, A., Arch. internat. de pharmacodyn. et de thérapie, **38**: 57. 1930. Abst., Physiol. Absts. **16**: 131.

Even large quantities of folliculin (up to 76,000 mouse units in the course of three weeks), which provoke remarkable changes in the uterus of the young cat, are incapable of altering the ovaries, which remain undeveloped. It is very probable that the reactivation of senile ovaries after injection of ovarian and placental extracts described by certain writers is due to accidental substances in the extracts and not to ovarian hormones.

The effect of the endocrine glands upon the sulphur metabolism as found in the urine of dogs. V. The influence of the ovaries (Ueber den Einfluss der endokrinen Drusen auf den Schwefelstoffwechsel im Harn des Jundes. V. Mitteilung. Ueber den Einfluss der Ovarien). Tasaka, S., Folia Endocrinol. Japon. **6**: 108. 1931.

In order to ascertain the influence of the ovarian parenchyma and the corpus luteum of the cow upon the sulphur metabolism the author fed several healthy grown bitches with a special ration until a nitrogen and sulphur equilibrium was established. The animals with continued corpus luteum feeding increased in body weight, and the urine volume diminished. The total nitrogen and total sulphur excretion in the urine was decreased, the latter somewhat

more than the former, so that the nitrogen sulphur quotient showed an increase. The ethereal sulphuric acid was decreased relatively the least, the neutral sulphur next and the inorganic sulphuric acid the most. The body weight of the animals decreased with continued ovarian feeding, while the urine volume showed no change. The total nitrogen and total sulphur excretion in the urine was slightly increased, the sulphur somewhat greater than the nitrogen. After stopping the ovarian parenchyma feeding the total sulphur output diminished more rapidly than the total nitrogen. The neutral sulphur was increased the most, the ethereal sulphur next, and the inorganic sulphur the least.

—J. Gagnon.

Relation of secondary sex characters to sex hormones (Die Abhangigkeit der formbildenden Reaktion von der Reife des somas und der Anwesenheit des Geschlechts-hormones). Mitzkevitch, M. S., Endokrinologie, **6**: 167. 1930.

The nipples of guinea pigs were used as indicators. Ovaries transplanted to castrated males produced development of nipples practically as great as in the normal female. The development of secondary sex characters is a double reaction based upon the age of the soma which permits the sex gland to produce the hormone which in turn compels the development of the nipple.—B. C.

Investigations on the female sex (Untersuchungen über das weibliche Sexualhormon). Wieland, H., W. Straub and T. Dorfmüller, Ztschr. f. physiol. Chem. **186**: 97. 1929.

By vacuum evaporation, extraction with benzine and ether, removal of impurities by washing with alkali, and treatment with acetone and petroleum ether, a crystalline but impure preparation of female sex hormone was obtained, which assayed 2000 mouse units to the milligram. Using the material so obtained, a yellow color, insoluble in chloroform, appeared when the Salkowski reaction was tried, and an orange yellow, with green fluorescence with the Liebermann-Burchard test. The results with these tests resemble those obtained with the gallic acids rather than with sterols. An alternative procedure, using the principle of the increased solubility of neutral organic substances in solutions of the alkali salts of gallic acids, yielded a non-crystalline product of the same estrogenic value.—S. Walker.

The problem of sex differentiation in the chick embryo with reference to the effects of gonad and non-gonad grafts. Willier, B. H., J. Exper. Zool. **52**: 65. 1928.

A total of 942 embryos was examined to ascertain the effects of gonad and non-gonad grafts upon sex differentiation in the chick embryo. Of these (a) 190 were hosts to grafts of gonads, obtained chiefly from the chick embryo and in a few cases from the adult rat; (b) 226 embryos were hosts to grafts of non-gonad tissues, such as the thyroid, thymus, hypophysis, liver, spleen, muscle, and kidney. These tissues were obtained chiefly from adult and in a few cases from juvenile chickens; (c) 423 were normally incubated, and (d) 103 embryos were subjected to the operative technique without grafting or to altered humidity. The embryos were incubated for 16, 17, 18, or 19 days, the great majority for 18 days. Several breeds of the common fowl were used. Embryos with either typical or atypical genital systems were found in all groups regardless of whether they were experimental or control and whether they were hosts to gonad or non-gonad grafts. In the typical female the right oviduct had disappeared with the exception of a duct-like portion attached to the cloaca and designated "the cloacal stump." It was approximately 4 mm. in length and without a shell gland. In the atypical female the right oviduct tended to persist so that a complete range was found between a typical cloacal stump and a right oviduct which was as completely developed as the left. The right ovary was always present and exhibited considerable variation in size. In a few of the larger ones, a cortex which was macroscopically visible had developed. The left ovary and oviduct were very rarely atypical. In the typical male the oviducts were absent; in the atypical males, both the right and left oviducts tended to persist. For each a complete range was found between a short, thread-like trace to an oviduct which resembled, to the extent of being full length and having an ostium, a shell gland, and rudimentary ligaments, the left oviduct of the female. The testes, although showing considerable variation in size and form, were regarded as typical in the majority. In a few cases they were sufficiently flattened and different in size to resemble right and left

ovaries. In a small number of embryos the testes were more or less covered with a black pigment. The distribution of the pigment was peculiarly related to the anteroposterior axes and the laterality of the testes. Pigmented testes occur only in certain breeds of fowls. The atypical genital structures of the embryos which were hosts to gonad grafts were identical with those found in the embryos with non-gonad grafts and in the normally incubated embryos. Atypical genital structures of exactly the same kind may appear in the host embryo regardless of the sex of the grafted gonad. The majority of the embryos, regardless of whether they were hosts to ovarian or testicular grafts, had typical genital systems. Operative technique alone or altered humidity increased the number of the atypical structures, but not as much as the grafting of tissues to the chorio-allantoic membrane. The increase was from 18 per cent for the normally incubated to over 24 per cent for the embryos subjected to operative technique without grafting or altered humidity, and to over 34 per cent for the host embryos. These results suggest that such conditions as low temperature and perhaps manipulation, to which the egg is subjected in the operative technique and especially in grafting, favor atypical sex differentiation. Low temperature, which, in general, is known to have a retarding effect upon developmental processes, apparently inhibits the retrogression of both müllerian ducts in the male and of the right müllerian duct only in the female. Thus these ducts persist in various stages of degeneration. Certain new experiments indicate that chilling of the egg for a short time on the eighth day of incubation, the time when retrogression begins, will greatly increase the number of embryos with atypical oviducts. The results as summarized above demonstrate that chorio-allantoic grafts of gonads (chiefly of the chick embryo) do not specifically cause atypical sex differentiation in the host embryo. Furthermore, these results do not support the conclusion of Minoura that sexual differentiation of the host embryo is modifiable by sex hormones from gonad grafts. The negative evidence given in this paper for the action of sex hormones in the embryonic differentiation of sex characters in the chick is discussed and recognized as furnishing a problem for further analysis.
—Author's Abst.

The heart hormone (Über das Herzhormon). Haberlandt, I., Endokrinologie, **6**: 335. 1930.

Substances from the frog heart give similar results to those of the heart hormone of higher animals. The substance is soluble in absolute alcohol, slightly soluble in chloroform, insoluble in ether, capable of dialyzing, and thermo-unstable. It is probably neither fatty nor albumenoid. The therapeutic use of the heart hormone is discussed.—B. C.

The presence in human urine of a substance having the same action on the thyroid as anterior lobe extract and the interpretation of the reaction for the diagnosis of pregnancy (Sur la présence dans l'urine humaine, d'une substance douée de la même action sur la thyroïde que l'extrait préhypophysaire, et sur l'interprétation de la réaction de diagnostic de la grossesse). Aron, M. and M. Klein, Compt. rend. Soc. de biol. **103**: 702. 1930.

In rabbits and guinea pigs subcutaneous injections of human urine from whatever source produce signs of activity in the thyroid resembling those following injections with anterior lobe extract. Since this thyroid stimulating substance is generally distributed and the substance producing the Aschheim-Zondek reaction is confined to the urine of pregnant women, it is not anterior lobe substance which is responsible for the latter reaction. It is more probably a product of the placenta.—J. C. D.

Basal metabolism in hypophysectomized dogs (Metabolismo basal en los perros hipofisoprivos). Artundo, A., Rev. Soc. argent. de biol. **6**: 497. 1930.

A lower basal metabolic rate was found in 5 of 8 dogs. The average for the 8 was —15.8 per cent (from 0 to —32 per cent). The average per sq.m. per day was 666 calories in operated and 795 in normal animals. The respiratory quotient was the same in both series.—J. T. Lewis.

Specific dynamic action in hypophysectomized dogs (Acción específica dinámica en los perros hipofisoprivos). Artundo, A., Rev. Soc. argent. de biol. **6**: 507. 1930.

The same values were found as in normal controls.—J. T. Lewis.

Early diagnosis of pregnancy: experience with the Zondek-Aschheim test. Parvey, B., New England J. Med. **203**: 1121. 1930.

A brief but comprehensive illustrated account of the history and methods of this test. In 256 private patients, the author found an accuracy of over 95 per cent—J. C. D.

Hypophysis and chromatophore reaction (Hypophyse und Chromatophoren reaktion). Bayer, G., Endokrinologie, **6**: 249. 1930.

Description of a "white" frog is given in which the lack of color was not due to a loss of pigment, but rather to the continued contraction of the pigment cells. Sections of the hypophysis showed a cyst-like formation replacing the pars intermedia. Since melanophore expansion and contraction were absent it is assumed that the pars intermedia secretes or liberates a melanophore activating principle.—B. C.

An experimental study of the growth effects of the anterior lobe of the hypophysis on the teeth and other tissues and organs. Downs, W. G., Jr., J. Dental Research, **10**: 601. 1930.

Observations were made on the chemical content and histological changes in the tissues of mice on pituitary medication and of the effect of this medication on the teeth and jaws, both grossly and microscopically. Gross and microscopic examinations were made of the effects of pituitary medication and hypophysectomy on the teeth, jaws and viscera of dogs. The conclusions were, that the pituitary had a definite effect on the growth of animals causing increased water retention in the tissues, increased calcium retention and decreased fat formation. It influenced the development of teeth and jaws in the direction of accelerating the growth of the teeth and somewhat increasing the size of the maxilla but did not cause malformations of any sort or absence of teeth in either under- or over-secretion. A comprehensive bibliography of the endocrine and metabolic literature as applied to the dentition, is appended.—Author's Abst.

Experimental contribution to the vital function of the hypophysis (Experimentelle Bidrag Til Sporgsmaalet Om Hypophysens Livevigtighed). Felding, S., Bibliot f. laeger, **121**: 483. 1929.

The author reviews the anatomy and physiology of the hypophyseal-tuberalis region, and gives a resumé of the results of the experimental researches obtained up to the present time. The author has used a technic of operation through the transphenoidal sinus, by means of which he has carried out several mechanically different operations on 35 dogs. These operations were supplemented in 24 cases by the injection of formalin into the hypophysis, for the purpose of destroying this gland. The results of this method of extirpation as well as the results of his methods of destruction agree well with each other. Ten dogs were operated on as controls and 17 dogs were rejected because of accidental conditions which made them useless for the evaluation of these problems (the occurrence of meningitis, hemorrhages and accidents of preparation). The anterior lobe does not appear to be necessary for life and the author believes that if its partial or total separation causes death the latter is due to a lesion of the tuber basal; it must be emphasized that the presence of even half of the anterior lobe could not prevent the fatal progress when there is a lesion of the tuber basal. The significance of the intermediate part (and of the pars tuberalis) is still a little doubtful, for total ablation of these cells has scarcely ever been accomplished without lesion of the tuber basal; but it is very probable that they are not of vital importance, because the greater part can be extirpated without consequences when the tuber basal remains intact. If there is a lesion of the tuber basal—whether the hypophysis is extirpated or destroyed or not—death is produced in the typical manner formerly attributed to lack of the hypophyseal hormone (and especially that of the anterior lobe). After the end of narcosis during a period varying from several hours to several days, apparently normal conditions prevail which are followed by apathy, drowsiness, coma and death. In cases where the lesion exceeds the tuber basal the natural awakening after narcosis does not take place, the condition being either cachectic or comatose, leading to death after a lapse of more or less time and very frequently accompanied by considerable drops of temperature. If the tuber basal is intact a long survival period

ensues even after the most varied experimental operations in this region. It has been determined that a small tuberienne lesion is not absolutly fatal. The formation of a clot at the base of the third ventricle seems to be of more importance—the latter applies especially to cases where injections of formalin into the hypophysis have been made—but as there are cases of lesion of the tuber basal after the injection of formalin into the hypophysis having the same fatal progress as is the case with clots, the lesion of the tuber basal would certainly be responsible for the fatal progress. Moreover the author has observed a case of a long survival in spite of the presence of a clot in the tuber basal. While the lesion of the tuber basal appears to be responsible for the experimentally produced death, death has formerly been attributed to lack of the hypophyseal hormone. Minute physiological examinations will be necessary to explain the mechanism of this death. The author calls attention to a possibility of a parallelism between the type of death ascribed above and often inexplicable occurrence of death in man following operations for cerebral tumors localized in a non-vital region. In these cases the author proposes the microscopic examination of the tuber basal since the presence there of a necrosis or of a hemorrhage produced either by pressure or by traction could have escaped the examiner in even a very careful autopsy. Until a negative result of such an examination has been given, the results of the present work seem to call for a little reserve in regard to operations on tumors in the hypophyseal tuberalis region, since the results obtained by x-ray and radium therapy seem to equal those obtained by means of operations up to the present time. Also in the other cerebral operations our knowledge of the danger through lesion of the tuber basal should be a warning to proceed with caution.—J. Gagnon.

The diagnosis of pregnancy by the Aschheim-Zondek test. Finkel, H. S., New England J. Med. **204**: 203. 1931.

This is a study on 52 cases in which the initial diagnosis was obscure. The test was positive in twenty cases, all of which were later proved pregnant. It was negative in 32, of which 31 were not pregnant. The one remaining had a "blighted ovum." Case histories and charts together with a brief account of the test are given.—J. C. D.

Morphology of the rat after iodine-poor feeding (Zur Jodmangelltheorie des Kropfes Morphologie der Rattenschilddruse nach jodarmer Ernahrung). Hellwig, C. A., Endokrinologie, **6**: 161. 1930.

Experimental rats after 119 days' feeding of diets poor in iodine show degeneration, especially of secreting cells. Comparative studies of colloid, follicle, stroma, etc., give results as expected.—B. C.

On the clinic and diagnosis of Simmond's disease (Zur Klinick und Diagnose der Simmondsschen Krankheit). Herman, K., München. med. Wchnschr. **77**: 1620. 1930.

The writer reports a patient, 48 years old, who had suffered for some time with her stomach, anorexia, vomiting, pain in the epigastrium, fullness and a feeling of heaviness. The same condition had existed formerly but disappeared after a short time. The physical examination was negative except for emaciation, pallor, listless expression, easily wrinkled skin, bad teeth, and moderately painful epigastrium on pressure. A test breakfast revealed complete anacidity. The stool was normal. The blood picture showed hemaglobin, 60 per cent; R.b.c., 4,000,000; W.b.c., 9,000. An x-ray examination of the gastro-intestinal tract gave negative findings. Her physician had sent her for operation with diagnosis of carcinoma. This diagnosis was ruled out because of the negative x-ray findings. He had given her hydrochloric acid for the anacidity but the vomiting and emaciation continued. The possibility of anaemia, pernicious anaemia and parasites was considered, but the characteristic blood picture was lacking and no parasites were found in the stool. Luetic cachexia was also considered but the Wassermann test was negative. Another consideration was cachexia of unknown origin such as follows Hodgkin's disease, and metastases of malignant tumors. Another disease which had to be taken into consideration was Simmond's disease. Re-examination revealed very bad teeth and high grade alopecia, which are found in Simmond's Disease. This disease is really cerebral atrophy resulting from hypofunction of the anterior lobe of the hypophysis. As the patient's symptoms coincided with those of Simmond's Disease she was given anterior pituitary lobe and

hydrochloric acid. Vomiting ceased, the appetite improved, weight increased rapidly and the patient felt better in the course of four months. The author concludes that one should suspect Simmond's Disease in cases where there are gastric, hematologic and endocrine symptoms, and where the disease responds favorably to specific medication and the x-ray findings are negative.
—H. G. Beck.

Studies in ovulation. IV. Induction of ovulation in the hypophysectomized rabbit by administration of anterior lobe extracts. Hill, Margaret and A. S. Parkes, J. Physiol. **71**: 36. 1931.

Acid-alcohol extracts of anterior lobe produced ovulation in hypophysectomized rabbits, but only after a longer period than the normal reaction time after copulation.—C. I. R.

Studies in ovulation. V. The action of the ovulation producing substance of urine of pregnancy on the hypophysectomized rabbit. Hill, Margaret and A. S. Parkes, J. Physiol. **71**: 40. 1931.

Ovulation was induced in only 4 out of 19 hypophysectomized rabbits. Active extracts were prepared from the placentae of rabbits previously injected with urine, while extracts of normal rabbit placentae were ineffective.—C. I. R.

Action of mammalian anterior hypophyseal extract on the egg laying of batrachians (Action d'un extrait d'hypophyse antérieure de mammifère sur la ponte des Batraciens). Kehl, R., Compt. rend. Soc. de biol. **103**: 744. 1930.

In the amphibian discoglossus, daily intramuscular injections were given to animals captured in December and January. Egg laying occurred very soon in these, although controls did not lay until later in the season.—J. C. D.

Cephalo-thoracic lipoid dystrophy (Über die progressive Lipodystrophie [Lipodystrophie cephalo-thoracica]). Marañon, C. and A. Cascos, Endokrinologie, **6**: 87. 1930.

This condition seems to be connected with disease of the hypophysis and with a hyperthyroid state. It is due to the loss of ability to lay down fat by the cells in the upper part of the body. The difference between the fats appears quantitative rather than qualitative. The condition is exceedingly rare in man.—B. C.

Disturbance in regulation of circulation: A new symptom in insufficiency of anterior lobe of hypophysis (Störung der Kreislaufregulation ein neues Sympton bei Insuffizienz des Hypophysenvorderlappens). Schellong, F., Klin. Wchnschr. **10**: 100. 1931. Abst., J. A. M. A. **96**: 1446.

Schellong describes observations on 4 patients with hypophyseal insufficiency. The symptom to which he directs especial attention is a peculiar disturbance in the regulation of the circulation, which becomes manifest in collapse and fainting. The author points out that such conditions of weakness are mentioned in other case reports of hypophyseal cachexia, but they had never been completely explained. Because the attacks of fainting occur even after slight bodily exertion, the behavior of the blood pressure and of the heart action were tested during work. It was found that the blood pressure of these patients was not, as in normal persons, somewhat increased during exertion, but the systolic as well as the diastolic pressure was decreased, and the heart action did not show any changes. That this disturbance is not dependent on the height of the blood pressure during rest is proved by the fact that, even when the blood pressure had been increased by an injection of epinephrine, a considerable decrease followed the exertion. This regulatory disturbance in the circulation could be partially or wholly counteracted by the administration of preparations of the anterior lobe of the hypophysis. The author concludes that the hormone of the hypophysis influences the peripheral vessels. The decrease in blood pressure during bodily exertion in patients with hypophyseal insufficiency is probably a result either of a reduction in the peripheral resistance or of the abnormal dilatation of the peripheral vessels. That the atrophy of the anterior lobe of the phypophysis or that its insufficiency influences also the other endocrine glands has been proved by anatomic observations. The influence on other endocrine glands is the reason why one

frequently sees the signs of pluriglandular insufficiency. However, in spite of the fact that weakness and fainting are observed also in suprarenal insufficiency, the author thinks that in hypophyseal cachexia the changes in the blood pressure are not due to a disturbance in the suprarenals. He cites two factors that speak against this: (1) The blood pressure during rest is usually normal and sometimes even increased. (2) Epinephrine is not effective in counteracting the regulatory disturbance.

Sugar metabolism of the liver. I. Effect of adrenalin and insulin on the sugar given off by the liver and its glycogen content (Der Zuckerhaushalt der Leber. Der Einfluss von Adrenalin und Insulin auf Zuckerabgabe und Glykogengehalt der Leber). Molitor, H. and L. Pollak, Arch. f. exper. Path. u. Pharmakol. **154**: 280. 1930.

In a preliminary operation a liver lobe was fastened under the skin in dogs and rabbits. This allowed the extirpation of small pieces of liver on subsequent days without narcosis and without causing pain to the animals. "Liver blood" was obtained by cutting into the tissue and collecting the blood from the cut surface. In 34 experiments on dogs the average sugar content of liver blood was 110, of ear vein blood 107 mgm. per cent. The average blood sugar difference was therefore +3; the differences varied from +17 to —9 mgm. per cent. In rabbits the liver blood contained 7 to 30 mgm. more sugar than the ear blood. Injection of adrenalin was followed by an increase in this difference. In dogs the rise in blood sugar was moderate, even after subcutaneous injection of 5 mgm. of adrenalin. In some experiments adrenalin even failed to raise the blood sugar and in these cases the hepatic-ear blood sugar difference did not change. In rabbits the blood sugar raising action of adrenalin was much more marked and consequently the hepatic-ear blood sugar difference rose considerably, up to 71 mgm. per cent. In several experiments the total carbohydrates of the liver were determined according to the method of Lesser. A decrease was noted after adrenalin injection; the experiments extended for 15 to 120 minutes. After insulin injection the hepatic-ear blood sugar difference fell or did not change. The total carbohydrates of the liver had a tendency to decrease after insulin; an increase was never observed. It is concluded that adrenalin in the first 2 hours of its action in dogs and rabbits causes hepatic glycogenolysis, but no direct correlation between the height of the blood sugar and the loss of liver glycogen was noted. Insulin inhibits hepatic glycogenolysis, an effect which may be noticeable 10 minutes after an intravenous injection of the hormone.—G. T. Cori.

Insulin—pharmacology and therapeutic uses. Various authors, Rev. franç. d'endocrinol. **9**: 43. 1931.

A notable series of abstracts of significant recent papers.—R. G. H.

Methods and results of diabetic treatment. Allen, F. M., New England J. Med. **203**: 1133. 1931.

The diet should be sufficient to maintain the patient at slightly below normal weight. Exercise decreases, while rest, overweight, and diets of high caloric value increase the demand for insulin. This is true, even when the extra energy is derived from fat. Under proper conditions there need not be an undue curtailment of carbohydrates. The insulin dosage should be adjusted in amount, time and number to be optimum for the individual patient. As yet the death rate from diabetes has not been materially reduced in spite of the undoubted advances in methods of treatment available.—J. C. D.

Initial insulin hyperglycemia (Hyperglycémie initiale insulinique). Bellido, J. M., Arch. internat. de pharmacodyn. et thérapie, **38**: 456. 1930.

The author injected insulin between the serous and mucus layers of the intestine in a dog, without at any time obtaining the ordinary hypoglycemic action of insulin. He attributes this effect to the possibility that either the small quantity of insulin injected was sufficient to cause the hyperglycemic effect but was incapable of causing hypoglycemia or that the substance causing hyperglycemia is not the true insulin and is more resistant to trypsin than insulin.—J. Gagnon.

Treatment of ulcers of the stomach by insulin injections (Traitement de l'ulcere de l'estomac par les injections d'insuline). Cade, A. and P. Barral, Rev. franç. d'endocrinol. **9**: 49. 1931.

Insulin injections have been used for the treatment of gastric ulcers by various authors since 1926. On a series of 25 patients showing the gastro-duodenal ulcer syndrome, Cade and Barral have used the same treatment. In a majority of cases they have observed a quick disappearance of the painful phenomena, but without being able to accomplish an absolute cure. It is well understood that patients showing an organic stenosis or a perigastritis could not be helped by insulin treatment, which, on the other hand, is contraindicated when there is a suspicion of neoplastic lesion. Weak doses of insulin are sufficient (15 units a day). The treatment is prolonged 20-25 days and then taken again some months later. In cases where the improvement is not satisfactory the injection of insulin can be preceded by the injection of 20-80 grams of glucose.—J. Gagnon.

The hypotensive hormone of the pancreas in therapy of the anxiety neuroses with vascular phenomena (L'hormone hypotensive du pancreas dans la thérapeutique des états anxieux avec phénomenes vasculaires). Cossa, P., Rev. franç. d'endocrinol. **9**: 51. 1931.

Various therapeutic methods have been tried for several months by using the hypotensive pancreatic hormone (recently extracted by Gley and Kisthinios and now sold as angioxyl) for treatment of anxiety neuroses. Several authors have tried this treatment and reported their results. The author reports 2 cases in which, due to this therapy, he has obtained quick relief from the anxiety phenomena in hypertensive patients without sequelae. On the other hand, it has been used on 3 melancholic anxiety patients without results. The 2 successful cases corroborate those reported by Targowla, by Schiff and Trelles, and by Tinel.—J. Gagnon.

The care of the surgical diabetic. Gilchrist, R., Edinburgh M. J. **38**: 30. 1931.

The author gives the following summary: The nature of the metabolism of the diabetic is briefly described in order to show how acidosis is produced. This is the greatest danger to which the surgical diabetic is exposed. An improper diet, lack of food, anaesthesia and infection all predispose to the production of acidosis. The appropriate treatment and prevention of this condition is outlined. Emphasis is placed on the important part played by sepsis in antagonizing the action of insulin, in precipitating acidosis and in being a frequent accompaniment of diabetic coma. The use of insulin and dietary measures to combat the production and excretion of ketone bodies are described in detail. Post-operative diets, which have been found useful in a number of conditions, are given. The response of a case of diabetic gangrene to dietetic and insulin treatment is recorded.—J. C. D.

Pancreatic extracts in cardio-vascular therapeutics (Les extraits pancréatiques dans la thérapeutique cardio-vasculaire). Giroux, R. and N. Kisthinios, Rev. franç. d'endocrinol. **9**: 53. 1931.

Pancreatic extract, freed from insulin, is to be recommended in the treatment of angina of the chest, of the arteries, and of certain forms of arterial hypertension. It has the advantage, because of its low toxicity, of allowing large doses to be used. It presents no contra-indications; from this viewpoint it offers indisputable advantages over insulin. Finally, the interesting results obtained allow us to think that in the future by continuing the treatment for months, and even for years, that perhaps a definite improvement of certain vascular conditions can be effected.—J. Gagnon.

Digestive anaphylaxis, of multiple causes, successfully treated by insulin (Anaphylaxie digestive de causes multiples traitée avec succés par l'insuline). Langeron, L., Rev. franç. d'endocrinol. **9**: 54. 1931.

Observations were made of a case of facial eczema caused at first by the ingestion of alcohol, sugar, and salt, and then progressively by most foods with the exception of meat and eggs. All the usual medicines were used without results. The author had recourse to insulin injections which quickly allowed the eating of the formerly noxious foods. This anaphylactic action of

sugar and salt has been noted only in rare cases and in this case the therapeutic activity of the insulin can probably be explained by the transformation in the body of substances absorbed in a noxious form due to imperfect assimilation.—J. Gagnon.

Insulin shock in cretins (Über Insulinschok beim Kretin). Lauterburg, W. Mitt. a. a. Grenzgeb. d. Med. u. Chir. **41**: 715. 1930.

A discussion is given of previous investigations referring to this problem. The examination of the blood sugar in cretins after feeding with 100 grams of glucose showed a curve with a retarded ascent and an especially retarded decline. The upper limit of the hyperglycemia was on the average somewhat less pronounced than with normal people. Both branches of the curve showed large individual variations. The same retarded decline of the hyperglycemia has been observed in one case of postoperative myxedema and in congenital athyreosis. The ascent of the curve was similar to the one of normals. After feeding with 100 grams of glucose and an injection of 15 units of insulin (Lilly) the cretins had violent toxic symptoms and the level of the blood sugar sank considerably below the one of normals, who remained perfectly well. A patient with hypothyroidism with a basal metabolic rate of —19 reacted like the cretins. There are several possible explanations for the retardation of the curve decline: (a) a defective influence of the thyroid gland upon the mobilization of the liver glycogen or in general upon the function of the liver in metabolism of carbohydrates, and therefore a great sensitiveness to insulin; (b) a hyperfunction of the islands of Langerhans, through that in turn a retarded absorption in the gastrointestinal canal, a retarded decline of the hyperglycemia and relatively small degree of hypoglycemia; accordingly a strong sensitiveness of the body to suddenly introduced exogenous insulin; (c) probably the two disturbances are interconnected, corresponding to the balance of thyroid and pancreas. The sub-function of the pancreas must be considered as functional, namely, as an adjustment to the reduced thyroid function for the preservation of the necessary balance between the two blood glands. The sensitiveness of the cretins is due to the low insulin level and to the reduced compensatory glycogenolytic effect of the thyroid upon the liver. A direct mutual influence of the hormones of pancreas and thyroid is impossible according to previous investigations. The cretins do not react essentially differently from normals to fruit sugar. With both this sugar and glucose, the hyper-glucemia is retarded.—H. M. Bosshard.

Function of the liver in diabetes mellitus. Meyer, E. L., Arch. Int. Med. **47**: 182. 1931.

A report is given of observations, clinical and laboratory, on 100 diabetic persons. Seven per cent showed both clinical and laboratory evidence of hepatic dysfunction, 28 per cent only laboratory data, 48 per cent no abnormal indications and 13 per cent only minimal readings. Age and length of uncontrolled diabetes furnishes most hepatic dysfunctions. The author concludes that the modern diabetic regimen materially aids in improving hepatic function and makes them better operative risks. He also advocates investigating all cases of hepatic dysfunction for diabetes.—H. J. J.

Influence of insulin on the glycogen content of the perfused rabbit liver (Die wirkung des Insulins auf der Glykogengehalt der perfundierten Kaninchenleber). Nielsen, N. A., Biochem. Ztschr. **230**: 259. 1931. Abst., Chem. Absts. **25**: 1909.

On perfusing the rabbit liver with defibrinated blood without insulin there is a gradual decrease in the sugar content of the blood and a corresponding increase in the liver glycogen. The deposition of the glycogen in the liver is not uniform. Addition of ⅓ to 20 units of insulin to the blood causes a rise in the blood sugar concentration, and a loss of glycogen in the liver. No effect was observed with amounts smaller than ⅓ insulin units.

Clinical and laboratory experiences with high carbohydrate—low caloric diets in the treatment of diabetes mellitus. Rabinowitch, I. M., New England J. Med. **204**: 799. 1931.

This is a careful analysis based on 164 cases. The diet contained 246 grams of carbohydrate on the average and yielded 1774 calories. The author

states that it appears reasonable to conclude that there is both clinical and laboratory evidence to support the view that diabetics are able to tolerate much more carbohydrate than they were given in the past, without the use of extra amounts of insulin, providing, in addition to the increased amounts of carbohydrate, the principle of undernutrition is applied in the treatment. That this new diet has fundamentally altered the metabolism of the diabetic is suggested from metabolism data. These include plasma cholesterol, urinary nitrogen and respiratory quotient determinations.—J. C. D.

Acromegaly with diabetes mellitus and xanthoma diabeticorum. Ralli, E. P., Arch. Int. Med. **47**: 329. 1931.

Ralli reports a case of acromegaly, severe diabetes and xanthoma diabeticorum in an Italian man. The xanthomata disappeared during the patient's previous stay in another hospital, but recurred in greater number after he left the hospital. They were located especially on the forearms and about the knees. The cholesterol content of the blood was exceedingly high at first, 5,900 mgm., but later it came down to 385 mgm. as a result of treatment with insulin and diet. Examination with the Roentgen rays revealed typical changes of acromegaly. The presence of a pituitary neoplasm was assumed to be indicated by a large sella turcica and by erosion of the clinoid processes. The author comments that the "difficulty in keeping the patient continuously sugar-free, even with fairly large doses of insulin, may be accounted for by the increased activity of the pituitary gland," and thus adds another insulin-resistant case of hyperpituitary disease to those listed recently by the reviewer.
—H. Ulrich.

The blood sugar in the portal vein during insulin hypoglycemia. Discharge of sugar from the liver (La glycémie portes et ses variations au moment de l'hypoglycémie insulinique. Recharge glycémique du foie).

Hyperglycemia following insulin in dogs under different conditions (depancreatization, starvation, starvation and phlorizine) (L'hyperglycémie insulinique chez le chien au cours de divers états expérimentaux [dépancréatation, inanition, inanition et phlorizine]). Rathery, F., R. Kourilsky and Y. Laurent, Compt. rend. Soc. de biol. **103**: 864; 881. 1930.

The blood sugar in depancreatized, starved, and starved and phlorizinized dogs is distributed in the same proportions during the maximum effect of insulin as in normal animals. The discharge of glycogen from the liver is not influenced by its glycogen content. In dogs under similar experimental conditions, the hyperglycemia immediately following insulin upsets irregularly the relationship between the blood sugar in different vascular areas.—J. C. D.

Hyperglycemia immediately following insulin and the variations of liver glycogen. Nature of the phenomena (Hyperglycémie insulinique immédiate et variations du glycogène hépatique. Nature du phénomène). Rathery, F., R. Kourilsky and S. Gilbert, Compt. rend. Soc. de biol. **103**: 1235. 1930.

Hyperglycemia immediately following insulin injections occurs in dogs irregularly and to a varying degree. It occurs in decapsulated dogs and is therefore not dependent on the adrenals. It is not directly related to the glycogen content of the liver for it occurs to an equal degree in normal animals, depancreatized animals, and animals which have had the liver glycogen depleted by starvation and starvation combined with phlorizine.—J. C. D.

Urticaria and insulin resistance with reference to the relation of the skin to the carbohydrate metabolism. Rudy, A., New England J. Med. **204**: 791. 1931.

The author summarizes as follows: (1) A case of diabetes mellitus with temporary resistance to insulin following an urticaria is reported. Under treatment with large doses of insulin (515 units in the first 24 hours) the patient improved: insulin was omitted on the eleventh day and the blood sugar has remained normal since then. (2) A review of the literature reveals similar cases with varying outcomes. (3) It is suggested that impurities still found in insulin, as now prepared, may be the possible cause of urticaria. (4) The theories on inactivation of insulin are described. (5) The relation of the skin to the carbohydrate metabolism and to insulin resistance is discussed.—J. C. D.

Effects of insulin and epinephrine on the blood acetone in fasting children. Salomonsen, L., Am. J. Dis. Child. **40**: 718. 1930.

In fasting children less than 3 years of age an investigation was made on the effect of insulin and epinephrine on blood sugar, on blood acetone and on the content of fatty acids in the blood plasma during the hours immediately following the injections. In 8 experiments it was found that insulin reduces the blood acetone but in decreasing degree according to the decrease of sugar in the organism. Epinephrine under the same conditions produces an increase in the blood acetone, the increase being greater in proportion as the organism is more poorly supplied with sugar. In one experiment insulin caused a distinct decrease in the content of fatty acids in the blood. In 5 of 6 experiments injection of epinephrine was followed by a rise in the quantity of fatty acids.
—M. B. G.

The utilization of carbohydrate by totally depancreatized dogs receiving no insulin. Soskin, S., J. Nutrition, **3**: 99. 1930.

Totally depancreatized dogs, in which the absence of islet tissue was subsequently verified by post-mortem examination, were maintained without insulin for as long as 4 weeks. The depancreatized dogs used in these experiments differed from most animals used for previous work of a similar nature, in that they were allowed, by the use of insulin, to make a perfect recovery from the operation and anaesthetic. These dogs, after the withdrawal of insulin, were kept on a protein diet and although they lost weight steadily, they remained bright and active throughout most of the experiment. After the first week or two of the experiment the respiratory quotient usually showed a progressive rise. It was sometimes maintained far above the "diabetic" level for as long as two weeks, and in some cases rose above 0.90. Determination of the CO_2 combining power or total CO_2 content of the blood at intervals, revealed very little fall in these values throughout the experiment. Two animals actually showed an increase in these values during the later stages of observation. The administration of dextrose invariably caused a temporary retention of CO_2. It is evident, therefore, that the "blowing-off" of CO_2 was not a factor influencing the R. Q. At a very early stage of the experiment, the second day or third day in some cases, the D:N ratio had usually fallen below 2.8:1, and might be maintained far below this level for almost a month. The ketosis as judged from urinary excretion, reached its height about the fourth or fifth day, then steadily declined. The administration of 50 grains of dextrose in addition to the regular diet resulted in the retention of considerable amounts of this sugar, which was not excreted on subsequent days. This retention of glucose was often as much as 50 per cent of the amount administered and in one case where 100 grams was given, the retention actually amounted to 68 grams. The sugar administration often caused a decided rise in the R. Q. and usually resulted in a sharp drop in the total ketone excretion and a definite protein-sparing action. Every criterion, upon which is based the hypothesis that carbohydrate is directly oxidized in the normal animal, was thus obtained in the totally depancreatized dog receiving no insulin. It is concluded: (1) As judged by the usually accepted criteria, there is no evidence that the diabetic organism suffers any impairment in its ability to oxidize carbohydrate. (2) The results obtained are incompatible with the "theory of nonoxidation," and furnish strong proof for the "overproduction theory" of diabetes. (3) The revision of the meanings given to the D:N ratio and the R. Q., which this and other work necessitates, is discussed.—Author's Abst.

Is diabetes caused by paralysis of the pancreatic filaments of the right vagus nerve? Tuttle, G. H., New England J. Med. **204**: 963. 1931.

The secretion of pancreatic insulin is stimulated nervously by impulses from the medulla transmitted by way of the right vagus, and hormonally by secretin from the duodenal mucosa. Hypoglycemia is kept in check by mobilization of sugar through secretion of adrenin. Diabetes can be explained by an interruption of the vagus pathway with a resulting lack of stimulus to the islet tissue. It is suggested that administration of secretin by replacing this lack through hormonal action would be of value. Clinical and physiological evidence is brought forward in support of the author's views.—J. C. D.

Hyperinsulinism. Tuttle, G. H., New England J. Med. **204**: 1039. 1931.

Insulin is produced outside the pancreas. Depancreatized dogs use sugar and therefore produce insulin. In certain cancer conditions there is persistent hypoglycemia without any evidence of overactivity of the pancreas. This type of case suggests a connection between this cellular insulin and the ability of cancer cells to use large amounts of sugar.—J. C. D.

Calcemia, parathyroidectomy, parathyroid hormone, vitamin D and the vasomotor reflexes of the carotid sinus (Calcémie, parathyroïdectomie, hormone parathyroïdïenne, vitamine D et réflexes vasomoteurs du sinus carotidien). Bouckaert, J. J. and P. Regniers, Arch. internat. de pharmacodyn. et de thérapie, **30**: 225. 1930. Abst., Rev. belge. sc. méd. **3**: 97.

The intensity of the vasomotor reflexes of the carotid sinus depends upon the quantity of blood calcium. The hypocalcemia caused by the injection of sodium oxalate or by parathyroidectomy determines the decrease and even the disappearance of the vasomotor reflexes of the sinus in dogs. The injection of a calcium salt or parathyroid extract and the ingestion of vitamin D by increasing the blood calcium restores the vasomotor reflexes.—J. Gagnon.

The influence of the thymus on growth under normal and abnormal conditions (Der Einfluss des Thymus auf das Wachstum unter normalen und anormalen Bedingungen). Asher, L. and P. Ratti, Klin. Wchnschr. **8**: 2051. 1929.

Thymic feeding or the injection of thymic extract promotes growth in young rats. Growing rats which were kept in the dark and fed McCollum's diet did not show either histological or clinical evidences of rickets if they were given 10 grams of thymus at the same time.—M. B. G.

Roentgenology of the thymus in infants. Pancoast, H. K., Am. J. Dis. Child. **40**: 452. 1930.

The width of the thymus as shown by sagittal roentgenogram, which is the evidence usually employed in interpreting enlargement, is not a good criterion. The only signs of enlarged thymus of any value are the following: (1) Lateral deviation of the trachea as shown by the sagittal view; (2) narrowing of the trachea during the inspiratory phase at the thoracic inlet, as shown by the lateral view; (3) too great a collapse of the trachea during the expiratory phase at the thoracic inlet by the lateral view; (4) buckling of the trachea in either phase as it passes over and behind the top of the wedge-shaped gland. The roentgenographic exposures should be made in the erect posture. Two exposures should be made in the sagittal direction, one during full inspiration and the other during full expiration and accurately timed. The arms should be held above the head and the head held straight and the body should not be allowed to slump. Two lateral views with the arms held down must also be made during two phases. Differential diagnosis must be made from foreign bodies, retropharyngeal abscess, laryngeal and tracheal stenosis, congenital heart disease, asthma, whooping cough, meningitis, recurrent laryngeal paralysis and relaxed soft tissues of the larynx and pharynx.
—M. B. G.

Observations on the thymus gland. Carr, W. C., Am. J. Dis. Child. **40**: 688. 1930.

In a paper read at the annual meeting of the American Pediatric Society in 1930 on observations on the thymus in the new-born, Carr stated that large mediastinal shadows in the new-born are not evidence that the symptoms are of thymic origin as such shadows are frequent in normal infants. Disappearance of symptoms is not a therapeutic test as they may disappear spontaneously. He believes, however, that the thymus is capable of producing symptoms by pressure on the trachea or bronchi. In discussion, Griffiths, Brennemann and Sherman stated that they believed that there are two types of cases, one due to hyperplasia of the gland with production of symptoms, and the second, sudden death in children without any apparent cause and with no previous history of such symptoms. Thymic death was possible and enlargement of the gland should be treated empirically.—M. B. G.

The relation of the thymus and sugar metabolism (Modificazioni della curva glicemica in rapporto all'azione de rimo). Piana, G. A., Pediatria **37**: 1013. 1929.

Blood sugar determinations were made prior to and following thymus extirpation in 10 young rabbits who were on a definite diet. On the basis of his results, the author concludes that the thymus in young animals regulates carbohydrate metabolism and is antagonistic to other endocrine glands, especially the pancreas. Extirpation of the thymus produces a marked reduction of blood sugar.—M. B. G.

Thymic shadows of new-born infants. Podlasky, H. B. and S. E. Kohn, Am. J. Dis. Child. **39**: 782. 1930.

Roentgenographic studies of the thymus were made on 100 new-born infants. Definitely enlarged shadows were found in 35, but none of the symptoms usually referable to thymic enlargement was evident in any of these. All infants were discharged from the hospital in good condition.—M. B. G.

Thymus and spleen implantation and calcium metabolism in rabbits (Thymus-Milz Implantation und Kalkstoffwecsel beim Kaninschen). Wu, J. H., Jahrb. f. Kinderh. **129**: 89. 1930.

Nitschke has shown that the thymus of the calf has 2 substances, one capable of lowering serum calcium and the other lowering serum inorganic phosphorus. Later he was able to isolate these 2 substances from the spleen and lymph nodes. The author found that in 7 out of 8 young rabbits of the same litter, thymus and spleen implantation produced a more marked lowering of the serum calcium as compared with control implantation of liver and kidney. The 3 very young animals displayed a much more marked effect than the half-grown. No similar effect was noted on the phosphorus. Results of these experiments confirm the previous work of Nitschke of hormonal regulation of calcium metabolism by lymphatic tissue, especially the thymus and spleen.
—M. B. G.

The use of barbituric acid derivatives in thyroid surgery. Barton, F E., New England J. Med. **204**: 757. 1931.

The author summarizes as follows: (1) Phenyl ethyl barbituric acid (luminal) is used very effectively in thyroid surgery, as a non-toxic preoperative sedative; its use decreases the necessary rest period and increases the effectiveness of the sodium amytal. (2) The use of sodium amytal, orally, as a "balanced anesthesia," with gas oxygen, places a patient in a low basal state, by reducing apprehension and nervousness. (3) It increases vital reserve and allows the patient to be under control at all times. (4) Sodium amytal is decidedly non-toxic, it causes a marked amnesia and postoperatively, it depresses the vomiting center.—J. C. D.

The effect of daylight on the thyroid in relation to basal metabolism (Der Einfluss des Tageslichtes auf die Rattenschildruse mit Berucksichtigung des Grundumsatzes). Bergfeld, W., Endocrinologie **6**: 269. 1930.

Experiments carried out at Freiburg indicated a definite relationship between the function of the thyroid of the rat and light; darkness interfering with proper functioning. Later experiments carried on at Bern, indicated the same differences, but to a much less degree. The author thinks the differences may be due to the quality of light, the Freiburg rats receiving more ultraviolet than those at Bern. Basal metabolism studies made of the Bern rats showed about 10% increase in O_2 consumption and CO_2 output in favor of the "light rats." The heat production was slightly higher, but not significantly so, in the animals in the dark. The thyroid effect is not transmitted through the sympathetic nervous system.—B. C.

Clinical experiments with thyroid in gynecic practice: Menorrhagia. Cooke, W. R., South. M. J. **24**: 20. 1931.

From a study of over 150 cases, the writer concludes: (1) Menorrhagia, sterility and abortion (separately or in combination) occur as symptoms; (2) a considerable percentage of these symptoms is apparently relieved by administration of thyroid, and by no other means as yet discovered; (3) the admin-

istration of thyroid in such cases does no harm especially (although not necessarily) when regulated by observations of the basal metabolic rate; (4) the percentage of reported success in this work demands further and more general clinical application.—J. C. D.

Gaseous exchange, especially in thyroid diseases (Beitrag zur klinischen Gasstoffwechselbestimmung insbesondere bei Schilddrusenerkrankungen). Gantenberg, R., Endokrinologie **6**: 81. 1930.

Basal metabolism is lowered in cases of obesity complicated with or related to arteriosclerosis, gonadal function and adiposo-genitalis. In certain cases obesity can not be accounted for on a basis of basal metabolism. The thyroid is thought also to control nitrogen metabolism. These conclusions are based upon myxedematous patients.—B. C.

Radiation treatment of toxic goiter. Gerber, J., New England J. Med. **204**: 95. 1931.

The author considers cases of exophthalmic goiter and toxic adenoma when the toxemia is moderate, the goiter not too large, and cardiac complications undetectable, as suitable for x-ray treatment as contrasted with surgical interference. Heavily filtered rays from high voltage machines given in 20% of an erythema skin dose at each treatment have been found to produce as good final results as surgical removal of the gland. The arguments against this method are considered and its advantages outlined.—J. C. D.

The function of the thymus in the light of organic reactions to abnormal body conditions (Die Funktion der Thymusdruse im Lichte der Organreaktionen unter anormalen Korperverhaltnissen). Hammer, J. A., Klin. Wchnschr. **8**: 2313. 1929.

On the basis of animal experiments, it cannot be shown that the thymus is necessary to either life or health. Some of the animals, however, evidenced an increased sensitivity to toxic and infectious states after thymus extirpation. Many toxic substances produce an increase in the number of Hassal's bodies, but it is difficult to state in which way the latter protect against infection, if they do. It is also not known what the influence of the thymus is on growth or on the development of the sex organs.—M. B. G.

Radium therapy of exophthalmic goiter and of other forms of hyperthyroidism (Ueber die Radiumbehandlung des Morbus Basedowii und der Hyperthyreosen). Högler, F., Wien. klin. Wchnschr. **44**: 180. 1931. Abst., J. A. M. A. **96**: 1744.

Högler reports that, of 306 patients with exophthalmic goiter or with hyperthyroidism who in the course of 12 years received radium treatment, 217 showed marked improvement, which in many cases was almost equal to complete cure. In 46 patients a slight improvement was noted. In 43 cases there was no effect whatever, but an exacerbation as the result of radium irradiation was never observed. If these results are compared with those effected by surgical treatment or by roentgen irradiation, it becomes evident that radium therapy is as effective as those methods. Moreover, it should be taken into consideration that, if the radium treatment is given properly and with careful control of the basal metabolism, there is absence of serious complications, life-endangering hyperthyroid reactions or fatal outcome, whereas, in roentgen therapy, and more often yet after operations, such complications have been observed. The author prefers radium treatment to roentgen therapy, particularly when it is desirable to avoid stronger reactions. He considers surgical treament necessary in cases in which roentgen or radium therapy has failed, and also in patients in whom for social reasons generally prolonged irradiation is not practical. Operative treatment is always necessary in cases in which the goiter produces stenosis. However, even in these cases a preliminary radium treatment is frequently helpful. In regard to adhesions that develop following radium irradiation, in those cases in which later surgical treatment became necessary it was found that the adhesions, if present, were usually slight, and, in contradistinction to the vascular adhesions that develop spontaneously or after roentgen irradiation, they did not contain much blood and thus did not complicate the operation.

Blood pressure before and after operation in hyperthyroidism. Hurxthal, L. M., Arch. Int. Med. **47**: 167. 1931.

Blood pressure determinations were obtained under basal conditions at the time of the basal metabolic rate, in over five hundred cases of hyperthyroidism. A section of these cases was compared with the blood pressure findings in non-toxic goiter under the same conditions. A comparison was also made with the blood pressures obtained under the usual conditions of clinical examination. The figures indicate that blood pressure determinations taken under the usual clinical conditions are higher than those taken under basal conditions. The blood pressure, also, is higher in toxic goiter than in non-toxic goiter. The average blood pressure also increases with age, and shows no constant relationship to the height of the basal metabolic rate, and the basal pulse rate. Observation of conditions before and after operation, and at various intervals during the following year on patients with toxic goiter, showed that there is a decrease in the pulse pressure in clinically well cases. This decrease in pulse pressure takes place at all ages, and in patients with or without hypertension. The difference in pulse pressure before and after operation is based on averages, but when individual cases were considered it was found that a small percentage had an increase in blood pressure, either systolic or diastolic, or both. Thus, hypertension associated with toxic goiter is not reduced to any greater extent than the blood pressure in toxic goiter without hypertension. When the average blood pressures of patients who have been operated on for toxic goiter are compared with the blood pressures in a group of patients with non-toxic goiter, there is very little difference. There also appears to be a very little difference between the average blood pressure determinations in patients with goiter and other groups of patients. Comparison of the blood pressures in toxic adenomatous goiter, and exophthalmic goiter, shows very little difference up to the age of 50. Thereafter, the average for the toxic adenomatous goiter is higher than the patients who have exophthalmic goiter. The number of cases in this group above 50 was too small to permit drawing reliable conclusions. The author concludes that hypertensive vascular disease, when found with goiter, is an independent condition probably not related to thyroid toxicity.

—Author's Abst.

Certain problems in the management of thyroid disease. Ledbetter, S. L., South. M. J. **24**: 284. 1931.

A digest of the author's experience with 500 cases.—J. C. D.

The possible significance of the thymus gland in the syndrome of hyperthyroidism. Margolis, H. M., Ann. Int. Med. **4**: 1112. 1931.

The thymus gland was studied in 55 cases of exophthalmic goiter and 30 cases of adenomatous goiter with hyperthyroidism. In all but 8 cases of exophthalmic goiter, some degree of hyperplasia of the thymic parenchyma was noted. The structure of 12 of these glands resembled those seen in infants. In 19 cases the hyperplasia was confined to the medulla. The size of Hassall's Corpuscles were markedly increased. In 35 cases, hyperplasia was present in the glands which had previously undergone varying degrees of involution. In the adenomatous goiter group, there were 16 cases of some degree of hyperplasia, though none exhibited the infantile type of thymus gland. The author believes that the degree of thymic hyperplasia is roughly proportional to the degree of susceptibility to the development of hyperthyroidism.—E. L.

Blood cholesterol values in hyperthyroidism and hypothyroidism—their significance. Mason, R. L., Hazel M. Hunt and L. Hurxthal, New England J. Med. **203**: 1273. 1931.

Cholesterol values on 70 patients with thyroid disturbances are reported. Hyperthyroid conditions tend to diminish the level of the cholesterol in the blood. The lowest values were found in extremely toxic patients. There was, however, no definite correlation between cholesterol value and the basal metabolic rate. True myxedema was accompanied by a markedly elevated blood cholesterol. Low metabolic rates, however, without clinical evidence of myxedema were accompanied by normal cholesterol values. Cholesterol values are a definite help in estimating the gravity of hyperthyroidism, and are important aids

in diagnosis and prognosis and in estimation of the response to treatment in patients with hypothyroidism. Possible coincidental effects of disordered cholesterol metabolism are suggested.—J. C. D.

Hyperthyroidism: A review of 270 hospital records. McLane, Evelyn G., Minnesota Med. **13**: 652. 1930.

This group of cases was treated at the New Asbury Hospital, Minneapolis, Minnesota. Eighty-three per cent were females, and 17% males, with an average age of males of 38.8 years, and of females of 39.4 years. Symptoms, as complained of in order of frequency, were—nervousness, palpitation, loss of weight, fatigue, excessive perspiration, unsteadiness, increased appetite, dyspnea, abdominal cramps, choking, edema of feet, cough, hot flashes, decreased appetite, nausea, diarrhea, decreased menstrual flow, insomnia, mania, epistaxis, pruritis, and headaches. Physical findings in order were—palpable enlargement of the thyroid, tremors, heart murmur, exophthalmos, lid lag, nystagmus, and increased reflexes. Pulse pressure averaged 76, systolic reading averaging 146. Body weight was generally low. Standard pre-operative treatment consisted of bed rest, Lugol's t.i.d., luminal at night, and bromides t.i.d. for two to three weeks. Basal rates which previously averaged plus 20 to 60 were markedly reduced in 75%, and moderately in the remainder. Complications encountered were of cardiac and pulmonary origin, and wound infections. Mortality was 2.3%, the complications in these cases being auricular fibrillation, collapse of trachea, air embolism, and toxic myocardia.—Author's Abst.

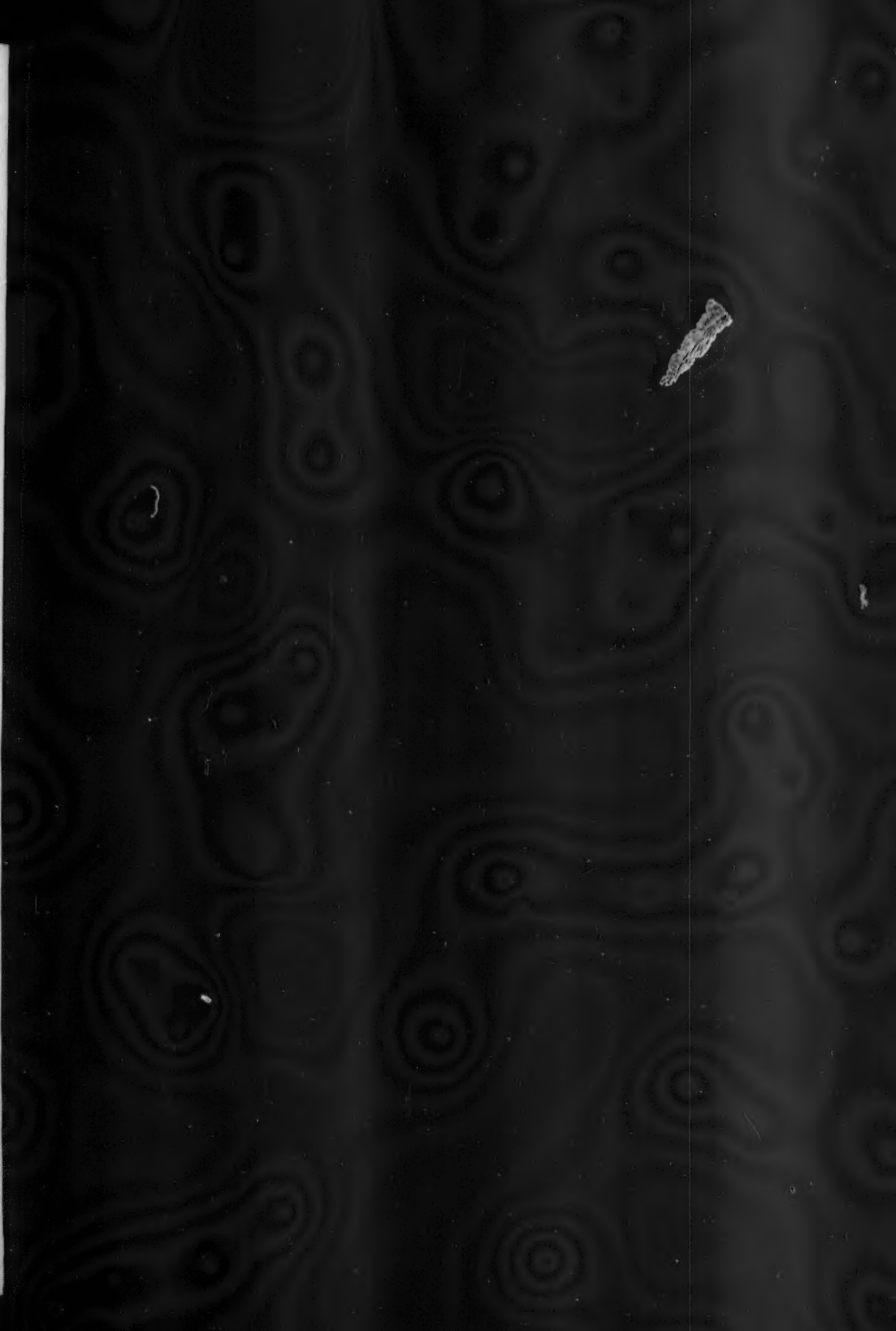
Interacinar epithelium of the thyroid gland. Moritz, A. R., Am. J. Path. **7**: 37. 1931.

This investigation of the mechanics of hyperplasia of and the significance of interacinar epithelium in the thyroid gland was conducted by means of serial sections and wax plate reconstructions. The study indicated that new follicles are formed by intra- and extra-follicular proliferation of the lining epithelium and that the secondary follicles may lose their parent connection and become isolated units. This was found to be true in the normal thyroids of fetuses, children and adults, in pathological glands the seat of hypertrophy and hyperplasia and in nodular goiter and in adenomas. In the scattered foci of hyperplasia in normal thyroid glands as well as in glands that were the seat of pathological hypertrophy and hyperplasia, labyrinthine intercommunication of acini was observed and was probably limited to the connection of primary with secondary acini. Solid masses of undifferentiated interfollicular epithelium were found in both normal and pathological glands, but such masses were readily accounted for as detached extra-follicular buds.—Author's Abst.

The iodine content of the bile and thyroid gland in cattle under the influence of the seasonal changes in feeding (Der Gallen- und Schilddrüsenjodgehalt bei Rindern unter dem Einfluss jahreszeitlicher Futterumstellung). Pfeiffer, G., Biochem. Ztschr. **230**: 290. 1931. Abst., Chem. Abst. **25**: 1880.

An increase in the average weight of the thyroid glands of cattle of various ages was observed from the winter months to the summer months. It has not yet been determined whether there is also a decline of weight from the summer to the winter months. The iodine content rises both with age and with the gland weight and reaches a maximum value in cattle 5-6 years old. The change from the iodine-poor winter fodder to the iodine-rich summer fodder has no recognizable influence upon the iodine of the thyroid glands. The bile secretion was found to increase with age. The iodine excretion in the bile during the summer is 20% higher than in winter, which shows that the iodine of vegetable matter is well absorbed by the animals. The iodine content of the feces cannot serve as an accurate guide to a study of iodine absorption.

Goiter and growth in height (Kropf und Längenwachstum). Stiner, O., Schweiz. med. Wchnschr. **59**: 1102. 1929.

On the basis of official reports, measurements of Swiss recruits, the author finds an average increase in stature of 2.2 cm. between the years 1888 and 1908, which increase is also to be seen in the following 20 years. Much of this is due to better living conditions, exercise and greater care of health, no one factor

being responsible. Cases of struma diffusa show quite consistently a greater stature, and probably more growth, than even normal cases or those affected with struma nodosa. The author thinks that any standards of thyroid size and activity need not have universal application, but individual and other circumstances would have to be considered. He considers his own data, and our general knowledge of thyroid, too meager to warrant definite conclusions, and seems rather skeptical of the virtues of iodine.—N. W. Ingalls.